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ARTERIO-VENOUS ANASTOMOSES IN THE HUMAN STOMACH

By T. E. BARLOW

From the Departments of Anatomy and Surgery, University of Durham, Newcastle upon Tyne

The precise distribution of the vessels of the stomach has been a subject of interest to anatomists and clinicians for many years. The common variations of the large arteries supplying this organ have been recorded, and the fate of the vessels after they pierce the muscular layer has been studied by dissection and by radiography.

These methods have shown that the branches of the large named arteries, on reaching the submucous layer of the stomach, break up into a network of channels readily visible to the naked eye, forming a free communication between vessels of the lesser and greater curvatures.

From this submucous plexus of large vessels finer branches arise which pass in an oblique direction through the muscularis mucosae to reach the mucous membrane (Disse, 1904; Reeves, 1920; Jatrou, 1920; Hofman & Nather, 1921; Djørup, 1922; and Babkin, 1940). The venous return is by comparable channels.

The recent work of Barclay & Bentley (1949) suggested that under certain conditions blood flowed directly from arteries into veins through arterio-venous anastomoses in the submucous layer of the stomach wall, so short-circuiting areas of the mucosa from the active circulation.

Arterio-venous anastomoses were first recorded by Lealis (1707), but they have only been regarded as of importance in the control of blood flow in comparatively recent times. Schumacher (1938) described two types of arterio-venous anastomosis. The first is a complicated knot of vessels ('glomus'), the walls of which are characterized by an epithelioid type of cell in the tunica media; these cells replace the typical muscle cell and are said to control the anastomosis by swelling or shrinking.

The other form exists as a sinuous but more direct connexion between artery and vein, and may be controlled by additional plain muscle cells in the tunica media.

These anastomoses are known to be present in many tissues, and there is reason to suppose that they exist in all organs, particularly where function is phasic in nature. Such an organ is the alimentary canal. Spanner (1932) has established the presence of arterio-venous anastomoses in the intestinal villi of man, and has also described arterio-venous anastomoses in the intestinal submucous plexus of other mammals, consisting of vessels arising from large submucous arteries and passing after a long course in the submucosa to venous 'nodes'. The 'nodes' may be in the form of 'rope-ladder' plexuses whose efferent vessels soon enter submucous veins. Spanner claims that the 'nodes' are dilated venous sacs, produced by contraction of muscle elements (sphincters) irregularly spaced along the vein wall.

METHODS

In order to study the precise arrangement of vessels in the human stomach wall, the vessels were injected with suspensions of red or blue pigment, the stomach wall was

cleared, and the vessels were dissected under the microscope. The stomachs were obtained from the cadaver, or at operations of partial gastrectomy for peptic ulcer.

Cannulae were tied into the right gastro-epiploic artery and vein and, in the case of an operation specimen, the cut edges of the stomach were secured by clamps to prevent escape of fluid. Leaking points were ligated, and the injection was continued until the small subserous and muscular branches were seen to be well filled on examination with a hand lens.

The injection mass consisted of 5 % red or blue pigment in 4 % gelatin solution; when capillary filling was not desired, 20 % of rice starch granules were added to the injection mass (Scharrer, 1940). The red or blue pigment is one normally used for colouring neoprene latex (B. B. Chemical Co. Ltd.). A mass of each colour was injected simultaneously into artery and vein respectively through a constant pressure apparatus at pressures not exceeding 150 mm. Hg on the arterial side and 80 mm. Hg in the vein.

After injection, the specimens were fixed for 24 hr. in 10 % formalin, with clamps still in position across the cut edges. The stomachs were then opened through the anterior wall close to the greater curvature, and portions were removed for clearing in 2 % potassium hydroxide solution. After 7 days the pieces were brought through successive strengths of glycerine into pure glycerine, in which they were kept.

The injected vessels were readily visible under the microscope in the cleared specimens, and by suitable dissection could be traced from the submucous plexus to the mucosal capillaries.

OBSERVATIONS

The arrangement of vessels in the submucous layer of the stomach wall is complex, but it is possible to describe two main systems of arteries and veins.

(1) Readily visible to the naked eye is a main submucous plexus of large arteries (Pl. 1, fig. 1) fed by the gastric and gastro-epiploic vessels, giving rise to branches which pass obliquely towards the mucosa and ultimately supply it. These are the mucosal arteries described by Disse (1904) and others. There is a corresponding venous plexus.

(2) Also lying in the submucosa is a second plexus of arteries and veins which vary in size from 100 μ to capillary diameter; it consists of two parts: (*a*) vessels which arise from and surround the main channels of the submucous plexus, some of the veins showing numerous dilatations which receive cross-branches from other veins; and (*b*) a series of arterial and venous loops of variable size (Pl. 1, fig. 2) which take origin from the main submucous vessels and spread their arcades throughout the submucosa. In places the loops end in capillaries within the connective tissue of the submucosa, but for the most part they remain as an intricate network, not penetrating the muscularis mucosae, though communicating with vessels in the muscular coat of the stomach. Numerous dilatations are seen in the veins of this connective tissue plexus.

Arterio-venous anastomoses

The arteries destined to supply the mucosa arise from the branches of the main submucous plexus and pass obliquely across the submucosa, branching and coiling as they reach the region of the muscularis mucosae, which they pierce to reach the

mucous membrane. From these oblique arterial vessels slender branches are occasionally given off which can be traced directly into neighbouring small veins which drain to the submucous plexus (Text-figs. 1, 2). In the dissections, the red (arterial) injection mass meets the blue (venous) about the middle of these anastomotic channels, but the proof of their arterio-venous anastomotic nature lies in the ability always to show a direct channel, nowhere less than 30μ in diameter, between an unmistakable artery and vein in the submucosa.

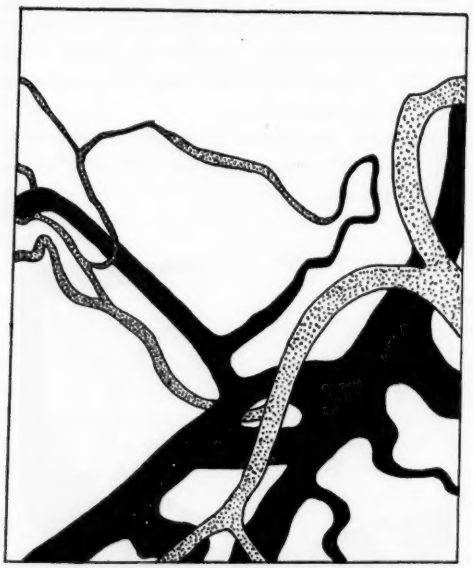
These anastomotic channels may follow a straight course, or a tortuous one, and may enter the parent artery at right angles or obliquely, against the direction of blood flow in that vessel (Text-fig. 2).

Text-fig. 1. Projection drawing of an Ektachrome transparency of an arterio-venous anastomosis in the submucosa (artery, stippled; vein, black). Arterial and venous branches of the submucous plexus cross the lower part of the field. Arising from the artery is a tortuous vessel which passes towards the centre of the field where it directly joins a channel which bends underneath it to reach an accompanying vein. (×32.)

The arterio-venous channel usually shows a narrowing at the point of junction of the red and blue injection masses, but the narrowest part in fixed specimens is never less than 30μ in diameter, and the general diameter is from 40 to 60μ. Recent observations by Walder (1950) suggest that many larger arterio-venous channels exist.

The complex arterio-venous anastomoses ('glomus') in the stomach wall described by de Busscher (1948) have not been observed with this technique. Only simple communicating channels between artery and vein in the submucosa have

been noted. The number, distribution and minute structure of these is yet to be determined.



Text-fig. 2. Projection drawing of an Ektachrome transparency of an arterio-venous anastomotic loop in the submucosa (artery, stippled; vein, black). The continuous channel can be readily followed between mucosal artery and vein. (×40.)

SUMMARY

The presence of arterio-venous anastomoses in the human stomach deduced by Barclay & Bentley (1949) has been demonstrated by double-injection technique. These are present in the submucous connective tissue of the gastric wall and are of the direct type.

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Fig. 1.

Fig. 2.

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EXPLANATION OF PLATE

Fig. 1. Three main arteries of the submucous plexus with the corresponding veins (artery, grey; vein, black). Mucosal branches can be seen leaving the main artery and proceeding towards the mucosa. (×6.)

Fig. 2. A loop of the extensive connective tissue plexus showing arteries and veins. Venous dilatations receive several smaller branches. (×32.)

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NEPHRIC DEVELOPMENT IN THE SHEEP WITH REFERENCE TO THE PROBLEM OF THE RUMINANT PRONEPHROS

By J. DAVIES
Anatomy School, University of Cambridge

The modern view of the mammalian pronephros is that it is merely the cranial part of a continuous excretory organ, and in particular that part which is directly related to the development of the Wolffian duct. The vestigial pronephros of the human is found at the level of the 7th to the 14th somites (Felix, 1912) with a high degree of individual variation. The pronephros of the cat has been studied by Fraser (1920) and shown to be at approximately the same level as in man. Fraser has reviewed the literature on the old controversy regarding the inter-relationship of the pronephros and the mesonephros, and has concluded that the two organs develop in a strictly cranio-caudal sequence and that no sharp dividing line can be drawn between them.

No account is available in the literature of the detailed development of the pronephros in even so familiar a mammalian embryo as the pig. The early excretory system of the sheep was studied by Bonnet (1888) and, as in Martin's account (1888) of the rabbit, the area of the developing excretory duct was called 'mesonephric'. Certain unique developmental features of the early excretory system, which have been described elsewhere (Davies & Davies, 1950), make a study of the sheep pronephros of unusual interest. The mesonephros in this animal was found to develop very early and to present a curious arrangement of the glomeruli and associated tubules in the area from the 7th to the 14th or 15th somites, that is to say in that part which in other mammalian embryos so far studied is considered pronephric. The glomeruli are fused in this zone into a continuous and irregular mass, called by these authors a 'giant glomerulus'. Its appearance is illustrated in Pl. 2, fig. 7, which may be contrasted with a similar section from a pig embryo of comparable age (Pl. 2, fig. 8). The glomerular complex is drained by about twenty-five short tubules, and merges caudally through a brief transition zone with glomeruli of the ordinary vertebrate type. Its early development was studied in detail, but due to the lack of suitable early stages the exact relationship of this part of the excretory system to the pronephros was not established. A number of early embryos have since been obtained and furnish the material for the present paper.

MATERIAL AND METHODS

Embryos of the following stages were studied: embryo 1, 7 somites; embryo 2, 11–12 somites; embryo 3, 14 somites; embryo 4, 23 somites; embryo 5, 29 somites.

The 14- and 29-somite embryos have already been described (Davies & Davies, 1950) and will be briefly reviewed. All the embryos, except the 29-somite specimen, were washed out with saline from the uteri of ewes killed at known stages of gestation.

They were fixed in half-strength Bouin's solution, cut serially at 5–6 μ and stained with haematoxylin and eosin.

DESCRIPTION OF THE EMBRYOS

Embryo 1 (*7 somites*)

The cranial 6 somites are united to the lateral plate by segmental cords of cells ('segment stalks') in which, however, no vestige of urogenital structures can be recognized. The intermediate cell mass opposite the 7th somite is unusually bulky and merges caudally with the unsegmented mesoderm. The intermediate cell mass, both at the 7th segment and for some distance caudally, presents a well-marked bulge dorsally (Pl. 1, figs. 2, 3). This is similar to that described in the cat embryo of 9–10 somites by Fraser, and called by her a 'pronephric ridge'. It will be referred to here simply as a 'nephric ridge' since its identity with pronephric structures is doubtful. The nephric ridge deeply indents the overlying ectoderm. The differentiation of the nephric ridge and of the intermediate cell mass outstrips that of the somites and extends for a considerable distance into the unsegmented part of the embryo. The intra-embryonic mesoderm is here very bulky, giving unusual dorsoventral depth to the embryo in transverse section. The intermediate cell mass appears in this region to be differentiated from the embryonic mesoderm by the development of a shallow dorsal groove by which it becomes demarcated from the unsegmented paraxial mass (Pl. 1, fig. 3).

Embryo 2 (*11–12 somites*)

The nephric ridge begins, as in the previous embryo, at the level of the 7th somite. No urogenital structures are present anterior to this, with the possible exception of a small but well-marked vesicle at the 6th somite on one side. It is connected to the coelomic epithelium but not to the somite.

The intermediate cell mass, beginning at the 7th somite, is quite continuous from this point caudally, its differentiation from the paraxial mass again extending well into the unsegmented part of the embryo. It is more bulky than in the previous embryo, contains many mitotic figures and is somewhat looser in texture. The connexion of the intermediate cell mass with the coelomic epithelium is less marked, though it is still continuous with the mesodermal core of the somite.

The nephric ridge now shows a further differentiation, namely the separation from its dorsal margin of a rod of cells (the Wolffian duct) and the appearance of vesicles (Pl. 1, fig. 4). About seven vesicles may be identified in the nephric ridge on each side of the embryo. They are not segmentally arranged and have no connexion with the coelomic cavity. They are in contact above with the primordium of the duct and merge ventrally with the intermediate cell mass. The duct primordium is not separated from the nephric ridge throughout its length but merges frequently with the dorsal wall of the vesicles or with the nephric ridge between them. The part of the nephric ridge which is cut off to form the duct becomes progressively more bulky in a cranio-caudal direction and at the same time shows fewer points of contact with the ridge and a greater degree of contact with the overlying ectoderm.

The duct primordium is closely applied to the ectoderm at about the level of the

future 14th somite and appears thereafter to be growing freely. It fades out in the ectoderm a short distance caudal to this level.

Embryo 3 (14 *somites*)

The intermediate cell mass is completely independent of the coelomic epithelium and may now be referred to as the nephrogenic cord. It is extremely bulky and by its bulging into the embryonic coelom causes a wide splaying of the somatopleuric and splanchnopleuric leaves of the mesoderm (Pl. 2, fig. 5). This is a typical feature of the sheep embryo in transverse section and contrasts with the conditions in man (Felix, 1912) and in the cat (Fraser, 1920).

The nephrogenic cord already shows intense angioblastic activity which, together with the formation of spaces by liquefaction of mesodermal cells, results in the development of the glomerular complex already referred to and described in detail elsewhere. About fourteen vesicles are present in the nephric ridge dorsal to the nephrogenic cord. The vesicles are not metameric and possess a basement membrane on all sides except ventrally. The Wolffian duct begins at a level slightly caudal to the second vesicle, a relationship which it maintains at its cranial end in later stages. It forms a continuous rod of cells clearly demarcated by a basement membrane from the nephric ridge and its contained vesicles. There are signs of lumen formation at several points. The duct is growing freely caudal to the 14th somite and ends in close apposition to the ectoderm some distance from the cloaca.

Embryo 4 (23 *somites*)

The size and extent of the primitive excretory organ at this stage are illustrated in Pl. 1, fig. 1. The allantois is a thick-walled bifid sac.

The excretory organ begins, as in all the previous embryos, at the 7th somite. The glomerular complex, lying approximately in the area from the 7th to the 14th or 15th somites, is almost fully developed. The epithelium over the glomerular septa is still cuboidal, however, and there are few connexions between the glomerular capillaries and the dorsal aortae. The associated tubules (about twenty-five) are short and entirely dwarfed by the massive glomerular complex ventrally. The cranial group of tubules opens directly into the Wolffian duct while in some cases the tubule seems to be replaced by a cleft in the glomerular mass (Pl. 2, fig. 6). The duct begins caudal to the second tubule, the first tubule emptying into the second and the latter into the cranial end of the duct. There are about forty-five tubules in various stages of development on each side of this embryo, the most rudimentary lying caudally. The Wolffian duct is patent throughout and opens into the cloaca.

Embryo 5 (29 *somites*)

The glomerular complex is almost fully developed and probably functional as judged by the histological and anatomical appearances. It is well supplied by endothelial outgrowths from the aorta or from its dorsal segmental and ventral intestinal branches. The cranial tubules already show an increase in length and an early differentiation into a 'secretory' and a 'collecting' segment. The arrangement of the first two tubules and their relationship to the Wolffian duct is the same as in

embryo 4. The epithelium investing the glomerular capillaries in the cranial half of the glomerular complex has been changed to a flat squamous layer. The allantois is now a thin-walled sac several inches long and contains a small amount of fluid.

Later stages

These have been fully described elsewhere (Davies & Davies, 1950). Pl. 2, fig. 7, is from an embryo of 4·5 mm. and 40 somites, and shows the general character of the glomerular complex and the simple nature of the tubules. In a pig embryo of a comparable stage of general development the cranial end of the excretory organ (mesonephros) lies some two or three segments caudal to that in the sheep. A 'giant glomerulus' is not found in the pig, Malpighian bodies of the ordinary type alone being found.

DISCUSSION

The development of the embryonic excretory organ in the sheep provides further evidence for the morphological unity of the pronephric-mesonephric system. The method of development of the Wolffian duct, namely by separation of a cord of cells from a continuous nephric ridge, closely follows the method described by Fraser (1920) in the cat. It differs in some particulars from the method demonstrated (Felix, 1912) in the human embryo, where the duct is developed by the caudal growth and mutual fusion of solid diverticula from the intermediate cell mass and more caudally from a continuous pronephric ridge. This method does not seem to be fundamentally different from that demonstrated in the sheep and cat, but is rather determined by the vestigial character of the area in man. It is of interest that, despite the rudimentary nature of the anterior nephric elements in most mammals, the duct continues to develop in association with them. Burlend (1931) has warned against reading too much into the cranio-caudal pattern of development of the embryonic excretory organ. It is no more remarkable than the cranio-caudal development of the vertebrae and the spinal ganglia, and cannot be regarded as necessarily indicating a phylogenetic sequence.

The area of the excretory system from the 7th to the 14th somites is not rudimentary in the sheep as in other mammalian embryos which have been studied. It becomes highly differentiated, with tubules and glomeruli, and persists until a late stage in embryonic life. It has none of the features usually considered as pronephric, such as coelomic chambers and funnels, and external glomeruli. The complex system of coelomic chambers described by Fraser in the cat is not found in the sheep. The sheep, therefore, and probably other ruminants (deer and cow, according to Bremer (1915)) which also possess a 'giant glomerulus' cannot be said to possess a pronephros in the accepted sense. The same area (7th-14th somites) in the birds and certain reptiles presents interesting points of comparison with the sheep. In these forms a pronephros and an extensive transitional zone are developed in this region, the latter containing fused external and internal glomeruli. The internal glomeruli undergo fusion and breakdown of intervening septa with progressive development to produce a glomerular structure not unlike the giant glomerulus of the sheep (Davies, 1950). The method of formation of the two structures is quite different, however.

The development of the giant glomerulus is probably determined by factors operating at the time of its appearance at an early stage. These are the association of an unusually bulky intermediate cell mass with a period of intense angioblastic activity in the embryonic mesoderm as a whole. The exuberance of the latter is perhaps a species-characteristic of the sheep. This is especially remarkable in the region of the septum transversum and of the primitive knot and is probably reflected in the development of other organ systems of this animal. The embryonic kidney becomes anatomically capable of function between the stages of 23 and 29 somites (16 to 17 days from copulation). The conditions are in contrast with those in other mammalian embryos about which accurate data are available. Thus in the cat the duct does not open into the cloaca until the 36-somite stage and the first glomeruli do not appear until about the same time (Fraser). The attainment of function in the embryonic kidney of the sheep coincides with the rapid and precocious expansion of the allantois and precedes the development of the foetal placental cotyledons by several days.

SUMMARY

1. The development of the early excretory system has been studied in sheep embryos of 7, 11, 14, 23 and 29 somites.

2. A continuous 'nephric ridge' is developed from the 7th to approximately the 14th somite and in relation to a bulky and precociously vascular nephrogenic cord.

3. The Wolffian duct is separated from the dorsal part of the nephric ridge as a cord of cells which grows freely caudal to about the 14th somite to reach the cloaca about the 19-somite stage.

4. Excretory tubules of a simple type are developed in the nephric ridge and become related ventrally to a large glomerular complex lying approximately from the 7th to the 14th somites, and continues caudally with glomeruli of the usual vertebrate pattern.

5. The significance of these findings in relation to the problem of the mammalian pronephros is discussed. A pronephros in the accepted sense cannot be said to exist in the sheep.

6. The embryonic kidney becomes capable of function, as judged by the anatomical appearances, at a very early stage (23–29 somites, 16–17 days). This coincides with the expansion of the allantoic sack.

The author wishes to thank Prof. H. A. Harris for his help and encouragement, and also Dr T. Ewer of the Institute of Animal Pathology, Cambridge, who made available the ewes from which the embryos were obtained. Thanks are also due to Mr J. A. F. Fozzard for the photographs, and Mr J. Simpkin and Mr J. W. Cash for technical assistance.

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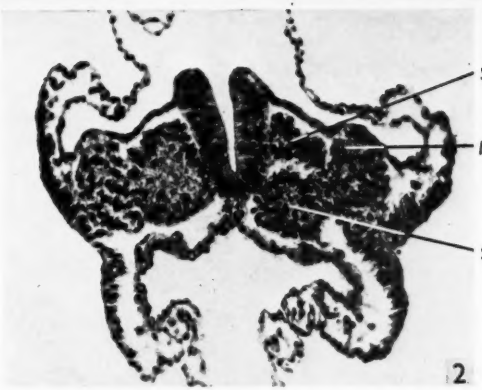
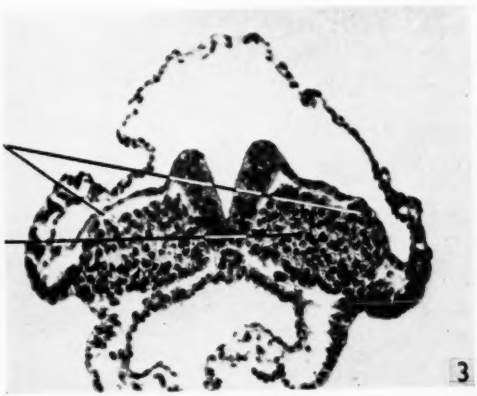
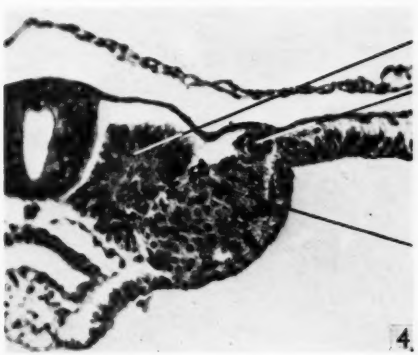
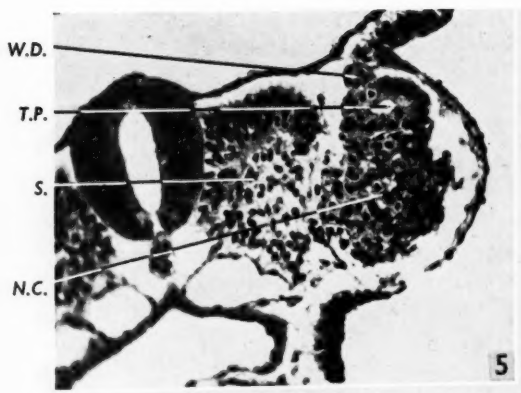






DAVIES—Nephric development in the sheep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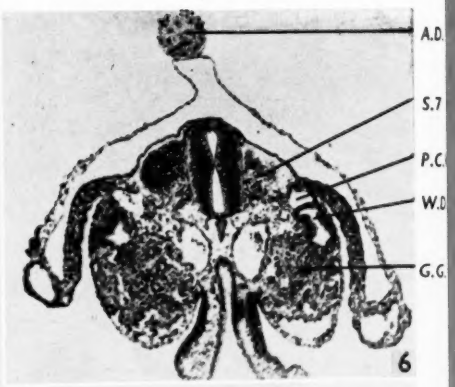
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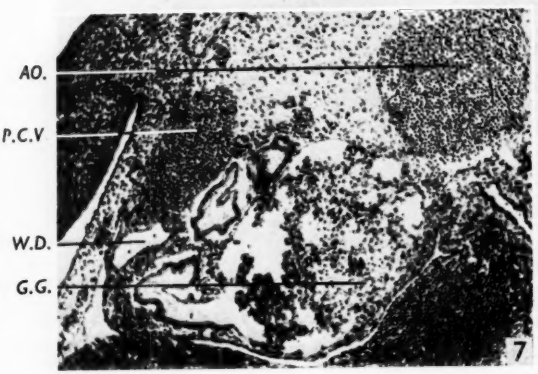
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EXPLANATION OF PLATES

List of abbreviations used in plates: *AO.* aorta; *A.D.* amniotic duct; *G.G.* giant glomerulus; *M.B.* Malpighian body; *N.C.* nephrogenic cord; *N.R.* nephric ridge; *P.C.V.* posterior cardinal vein; *S.* somite; *T.P.* tubule primordium or vesicle; *U.P.M.* unsegmented paraxial mesoderm; *W.D.* Wolffian duct.

PLATE 1

Fig. 1. Sheep embryo of 17 days and 23 paired somites seen from the ventral side. The Wolffian ridge extends almost the whole length of the dorsal body wall lateral to the developing mesentery. The allantois is bifid and thick-walled. The amniotic duct is seen as a fine thread dorsal to the embryo. ×7.

Fig. 2. Transverse section of sheep embryo of 7 paired somites passing through the 7th somite. The nephric ridge lies dorsal to the bulk intermediate cell mass forming a marked bulge in the ectoderm. ×175.

Fig. 3. Transverse section of same embryo as in fig. 2 but passing through the unsegmented part of the embryo caudal to the last somite. The nephric ridge is still visible. ×175.

Fig. 4. Transverse section of sheep embryo of 11–12 paired somites passing through the 8th somite. The intermediate cell mass is almost separated from the coelomic epithelium. The nephric ridge of the previous sections now contains a vesicle. The dorsal part of the ridge has separated as the primordium of the Wolffian duct. ×175.

PLATE 2

Fig. 5. Transverse section of a sheep embryo of 17½ days and 14 paired somites at the level of the 8th somite. The intermediate cell mass is now free of the coelomic epithelium and forms a bulky and already vascular nephrogenic cord. Dorsal to it is a well-marked vesicle and the solid primordium of the Wolffian duct. ×175.

Fig. 6. Transverse section of sheep embryo of 17 days and 23 paired somites (see Pl. 1, fig. 1), passing through the caudal end of the 7th somite. The tubules are short and open immediately into the Wolffian duct. Ventral to the tubules is the giant glomerulus permeated by irregular spaces and actively angioblastic. Notice the whorl-like remains of the amniotic duct, seen as a faint thread in Pl. 1, fig. 1. ×40.

Fig. 7. Transverse section of sheep embryo of 4·5 mm. and about 40 paired somites, illustrating the appearance of the giant glomerulus and the simple nature of the tubules. ×40.

Fig. 8. Transverse section of pig embryo of a comparable stage of general development as the sheep in fig. 7. The section passes through the first Malpighian body of the mesonephros. Compare the small size of the glomerulus at this level and the complicated character of the tubules with the conditions in the sheep (Pl. 2, fig. 7). ×40.

A COMPARATIVE STUDY OF THE VASCULARIZATION OF THE ADRENAL GLAND IN THE RABBIT, RAT AND CAT

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The work of Gérard (1913) and Anson, Cauldwell, Pick & Beaton (1947) has given a new insight into the vascularization of the human adrenal gland. While the source of the adrenal arteries from the inferior phrenic artery, aorta and renal artery, as described in contemporary textbooks of anatomy, is well known, it is not generally appreciated that the arteries from each source divide on approaching the adrenal, so that fifty or more arteries are to be found entering the periphery of the gland. Yet there is only one vein leaving it, which for the greater part of its length has only longitudinal smooth muscle in its coat (Brunn, 1873; Ferguson, 1906; Bargmann, 1933; Velican, 1948). This vascular arrangement is one which seems to be well fitted for ensuring an efficient blood flow through the adrenal. It is therefore important to assess the area of distribution of the arteries within the gland and the degree of anastomosis between them, for the efficiency of the arterial supply will depend on the functional capacity of this anastomosis.

Hoerr (1931) claimed that if all the blood vessels to the guinea-pig adrenal are lightly ligatured the gland atrophies fairly rapidly, being replaced by connective tissue at the end of 3 months. He observed, however, that if only the main blood vessels are ligatured, without disturbing any small capsular anastomoses, blood vessels from the latter will canalize the degenerating parenchymatous tissue so that the adrenal presents a surprising resemblance to the normal gland at the end of 3 months; but many cells fuse with their neighbours to form giant cells of fifty or more nuclei. Vogt (1947), in denervation of the rat adrenal, found it impossible to avoid injury to an artery; a small infarct at the cranial surface of the gland invariably resulted, and occasionally even atrophy of the whole gland.

Since these two observations are no more than occasional findings made in the course of other investigations, and pay no attention to the site of origin of the arteries interrupted or to their distribution within the gland, it was decided to examine first the detailed vascularization of the adrenal in one animal, the rabbit, and to determine the result of interruption of individual arteries. Subsequently a comparative study was made, under similar conditions, of the cat and the rat.

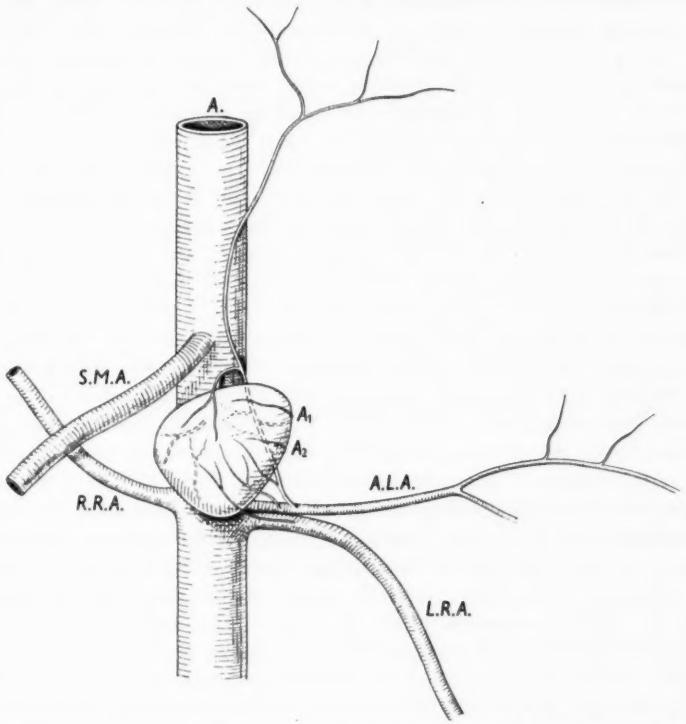
THE VASCULARIZATION OF THE RABBIT ADRENAL GLAND

Because of the easier access to the left adrenal in the rabbit, observations were confined to the gland of this side; although lying in the angle formed by the aorta and the left renal artery, and overlapping both, it is easily separable from them.

The arterial supply of the adrenal was observed in six rabbits by dissection, using a binocular microscope, after injection of a 10 or 20 % suspension of bismuth oxychloride ('Chlorbismol', May and Baker Ltd.) or 50 % barium sulphate ('Raybar cream', Damancy and Co. Ltd.) into the thoracic aorta. Neither of these suspensions

will pass through capillaries, owing to their particle size, and will therefore show only the arterial system of the adrenal when injected into the thoracic aorta. The finer intraglandular vessels were viewed in cleared serial sections of the adrenals of four rabbits after injection of doubly filtered india ink into the thoracic aorta.

The main source of arterial supply in the rabbit is the adrenolumbar artery, which arises from the aorta just above the renal artery, or from the renal artery



Text-fig. 1. Diagram of the vascularization of the left adrenal in the rabbit. The aorta (*A.*) is seen to give off the superior mesenteric artery (*S.M.A.*), right renal artery (*R.R.A.*), adrenolumbar artery (*A.L.A.*), and left renal artery (*L.R.A.*). The adrenolumbar artery gives origin to its adrenal branch, and then passes on to supply the body wall; the adrenal branch courses cephalically, deep to the adrenal, and ends by supplying the inferior aspect of the diaphragm. In its course it supplies small arteries which either enter the deep surface of the gland, or curve round its margins to enter it from the anterior surface. There are usually two arteries (A_1 and A_2) which curve round its left lateral border, and which are the easiest to approach and divide through an anterior incision in the abdominal wall. Separate adrenal arteries are also to be noticed arising from the aorta, renal artery or adrenolumbar artery itself.

itself. It courses under the lower pole of the adrenal, and here gives off its adrenal branch which runs deep to the gland in a cephalic direction and ends by supplying branches to the inferior surface of the diaphragm (Text-fig. 1). The other branch of the adrenolumbar artery, which is its continuation, passes out to vascularize the lateral body wall, and gives off an artery which passes caudally, deep to the kidney,

to vascularize perirenal tissues. One or two adrenal arteries are usually given off from the adrenolumbar artery before the main adrenal branch itself. The latter, in its course deep to the adrenal, furnishes a variable number of arteries (five to nine) which either enter the deep surface of the gland or curve around its caudal, left lateral, or cephalic border to enter it from its anterior surface. There are usually two arteries curving around the left lateral border, and these are the easiest to approach through an anterior incision in the abdominal wall (vide infra). The adrenal also receives two or three vessels directly from the aorta which either enter its deep surface, or curve round its medial border to enter its anterior surface, and usually one vessel from the renal artery which passes cranialwards, deep to the adrenolumbar vein, to run on to the anterior surface of the adrenal and enter it from there. The rabbit adrenal is therefore supplied by about ten arteries which approach it at various points around its periphery. There is, however, only one adrenal vein, which on the left side opens into the renal vein just after the adrenolumbar vein has joined the latter; on the right side it opens into the inferior vena cava.

The finer branches of the adrenal arteries ramify on the surface of the gland in the superficial part of its capsule, without forming any noticeable arterial anastomoses with one another. After perforating the capsule these vessels form a subcapsular arteriolar plexus from which capillaries pass into the substance of the cortex, or larger vessels (the 'arteriae medullae' of Flint, 1900) may penetrate the cortex without branching and terminate directly in the medulla. The cortical capillaries first form a meshwork in the z. glomerulosa, surrounding groups of glomerulosal cells; this plexus is formed only of capillaries and is continuous throughout the z. glomerulosa at the periphery of the cortex. From this glomerulosal plexus capillaries pass in radially towards the medulla between the columns of z. fasciculata cells, anastomosing only very poorly by means of sparse cross-communicating capillaries. In the z. reticularis the capillaries again form a plexus, in relation to the z. reticularis cells, and eventually open into the medullary sinusoids. The medulla is, therefore, supplied by its own vessels, the arteriae medullae, and by blood which reaches it at all points around its periphery through cortical capillaries. The former vessels are easily injected, whereas the latter were found to be either not injected at all, or only patchily, particularly when injection was made into an anaesthetized rabbit through the aorta. From the illustrations published by other authors (for example, Gersh & Grollman, 1941), it is obvious that similar difficulties have been experienced in obtaining a complete injection of the capillaries of the adrenal cortex in other animals. It may be assumed that the arteriae medullae provide the means whereby a blood supply to the medulla can be assured at all times in the presence of 'alarming stimuli' (Selye, 1946) independently of the capillary circulation through the cortex; in the cat, according to Bennett & Kilham (1940), the capillaries of the cortex can be narrowed by the distension of the cells of the outer z. fasciculata with lipoid, and this would similarly require an alternative vascular pathway to the medulla.

Occasionally, direct anastomoses of the branches of arteries on the anterior surface of the adrenal with small venous radicles draining tissues around the gland can be seen in the rabbit, as has been described in the cat by Bennett & Kilham (1940).

EXPERIMENTS

Materials and methods

Fourteen mature male and female rabbits, varying in weight from 1·0 to 2·4 kg., were used for the experiments. The left adrenal was exposed under nembutal anaesthesia through an abdominal incision parallel to and about 1 in. below the left costal margin. In the earlier experiments it was found necessary to inject the adrenal arteries with a 2% solution of methylene blue in normal saline at body temperature in order that they could be seen. This was achieved by injecting the methylene-blue solution into a plastic catheter of about 0·5 mm. internal diameter inserted into the carotid artery until its tip reached the aorta. This procedure was found to be unnecessary in the later experiments. The adrenal arteries were located with the aid of a binocular microscope after removal of the peritoneum over the surface of the gland. The artery to be interrupted was then picked up with fine forceps and a segment was removed with small scissors. In very few cases was it necessary to lift the adrenal from its bed during the procedure.

The rabbits were killed by means of an overdose of nembutal 3, 4, 5 and 9 days, and 2, 3, 4, 5 and 7 weeks after operation. After fixation in formol saline, serial sections of the adrenal were made and stained with Ehrlich's haematoxylin and eosin, Heidenhain's iron haematoxylin and eosin, Masson's trichrome or Mallory's (1936) method, a modified Wilder's technique (Lillie, 1948) and, in some cases, resorcin fuchsin.

Results

In every case a focus of necrosis was produced in the adrenal cortex. In no case was there any degeneration of cells in the medulla, although in one rabbit as many as four separate adrenal arteries were interrupted. The position of the areas of focal necrosis corresponds fairly closely to the position of the interrupted arteries in relation to the gland. Whenever more than one artery arising from the adrenal branch of the adrenolumbar artery is interrupted, only a single focus of necrosis is produced, which corresponds approximately to the sum of the areas of necrosis produced by interruption of each individual artery, and which has a greater tendency to include the z. glomerulosa. Interruption of the three separate branches from the aorta to the adrenal in one rabbit, however, produced three separate foci of necrosis. The detailed histology of the lesions is described below.

The one adrenal to which the three adrenal arteries from the aorta were interrupted was examined after 3 days and showed three foci of necrosis along the medial border of the gland, the middle focus being the largest. All the lesions involve mainly the z. fasciculata, the z. reticularis being normal and the z. glomerulosa being involved only slightly at some points. In the centre of each lesion the adrenal cortical cells are beginning to lose their outlines and their nuclei are less basophil than normal. At the periphery of the lesion some of the cortical cells retain their basophil nuclear staining, but most prominent are large numbers of densely packed basophil nuclear particles of very variable size embedded in eosinophil cytoplasm which shows no cell boundaries, suggesting that active karyorrhexis is proceeding. Large numbers of leucocytes are found scattered throughout the lesion, but par-

ticularly at the periphery. When treated by the method described by Romeis (1948), these cells are found not to be true eosinophils. The lesions are well differentiated from the surrounding cortex, since the compactness of the areas of focal necrosis with their accumulation of basophil particles contrasts well with the conditions in the surrounding z. fasciculata, where the cell columns are distorted and curved over the surface of the lesions. The capillaries in the remainder of the cortex are slightly distended compared with the normal adrenal.

The adrenal was examined in five rabbits 4 days after interruption of individual and separate arteries arising from the adrenal branch of the adrenolumbar artery or, in one case (rabbit 8), interruption of three such arteries and one artery arising from the aorta. In all cases the foci of necrosis involved only the z. fasciculata, neither the z. glomerulosa nor z. reticularis showing any degenerative changes (Pl. 1, fig. 1); in fact, the reticular appearance of the z. reticularis is exaggerated as a result of a dilatation of its plexus of capillaries. The one exception is the adrenal of rabbit 8, in which the z. reticularis is compressed against the medulla due to the large size of the lesion caused by interruption of the three arteries from the adrenal branch of the adrenolumbar artery; the surface of the lesion also includes the z. glomerulosa (Pl. 1, fig. 2). The interruption of the aortic branch in this animal produced a separate small lesion in the outer part of the z. fasciculata. In the centre of each focus of necrosis the nuclei of the cortical cells are seen to be losing their basophilia, but their cytoplasm is more eosinophil than in the remainder of the cortex. Some cells have become confluent and their nuclei have completely disappeared. Small irregular chromatin particles are scattered all over the lesion in between the cells, but at the periphery of the lesion in three rabbits there are, in addition, large numbers of basophil nuclear particles surrounded by scanty eosinophil cytoplasm which shows poorly defined cell outlines. These well-demarcated particles, which are usually of the same size and shape as normal cortical nuclei, or just slightly smaller, are intermingled with large numbers of much smaller, irregular, dust-like basophil granules. Around the periphery of each lesion there is a circumferential zone in which cortical cells have either died leaving only their reticular framework, or show pyknosis of their nuclei with complete loss of cytoplasmic staining. Leucocytes are also obvious in some cases, particularly in the part of the circumferential zone between the lesion and the z. reticularis. There is some dilatation of capillaries in the remainder of the cortex.

The appearance of an adrenal 5 days after interruption of two arteries of supply arising from the adrenal branch of the adrenolumbar artery is essentially similar to that at 4 days, except that almost the whole lesion is filled with the basophil nuclear particles already noted. Leucocytes are very prominent in the circumferential zone on the deep aspect of the focus of necrosis. The z. glomerulosa and z. reticularis are intact, but the latter is compressed against the medulla.

In one adrenal of which two arteries, arising from the adrenal branch of the adrenolumbar artery, were interrupted 9 days before death, the lesion is very like that at 5 days, having a profusion of basophil particles at its periphery. The cortical cells remaining in the centre of the lesion show marked eosinophilia of their cytoplasm and pyknotic or karyolytic nuclei. The circumferential zone is clearly marked, particularly over the major part of the capsular surface of the lesion. The z. glomeru-

losa cells appear quite intact over half of the surface of the lesion, although their cytoplasm is markedly eosinophil; in the other half of the lesion the area of necrosis reaches the surface and involves the z. glomerulosa. The z. reticularis is normal. In the other adrenal examined after the same post-operative time interval, in which only one adrenal artery was interrupted, the cortical cells resemble those in the circumferential zone of necrosis in previous adrenals, with very faint cytoplasmic staining and nuclei which are usually pyknotic but which may be apparently quite normal; occasionally the cells have completely degenerated leaving small spaces covered by a reticular framework. There is no accumulation of large basophil particles as in the previously described adrenal. The lesion is triangular in shape, with its apex abutting on the medulla. Over half of the surface of the lesion the z. glomerulosa cells are normal, but those over the other half of the lesion are quite dead, leaving only their connective tissue framework.

To follow the further course of histological changes, only one adrenal artery arising from the adrenal branch of the adrenolumbar artery, and curving round the left lateral border of the gland, was interrupted, as in the case just described.

At 2 and 3 weeks after operation the area of focal necrosis involves only the z. fasciculata (Pl. 1, fig. 3), and shows pronounced degeneration of cortical cells. Some cells have completely disappeared (particularly at the periphery of the lesion), leaving only empty spaces lined by reticular fibres; in the cells at the centre of the lesion the nuclei have usually disappeared and the cytoplasm of the cells has become confluent. A few small, irregular basophil particles are present in the centre of the lesion, where the network of argyrophil fibres normally present around the cells of the z. fasciculata is either fragmented or absent. At the periphery of the lesion the normal reticular framework seems to be preserved, the fibres being thicker than normal. A few macrophages, which are filled with basophil granular material, have appeared in the lesion three weeks after operation.

The focus of necrosis 4 weeks after operation is small and triangular in cross-section, with the apex of the triangle towards the medulla, the base of the triangle being depressed from the surface of the gland. The histological appearance suggests shrinkage due to fibrosis. Most of the glomerulosal cells over the surface of the lesion are intact (Pl. 2. fig. 1), but a few show pyknotic nuclei. The z. fasciculata cells within the lesion itself are either necrotic or show nuclei which are condensed and eosinophil, or which, while retaining some basophilia, are undergoing karyolysis. Blood vessels at the apex and at the capsular surface of the lesion are markedly dilated.

One week later the area of focal necrosis is even more contracted and depressed from the surface of the gland (Pl. 2, fig. 2), while the z. fasciculata cells within the lesion itself are only represented by a few condensed eosinophil nuclei in the meshes of a rich network of argyrophil fibres which bounds the numerous small vacuoles scattered all over the area of necrosis. The cells of the z. glomerulosa show pronounced changes. Their nuclei are losing their basophilia and some show karyolytic changes, while their cytoplasm is becoming confluent, so that the groups of glomerulosal cells appear as a syncytium (Pl. 2, fig. 3).

Seven weeks after operation the histological characteristics of the lesion are similar to those at 5 weeks, except that now there are no intact glomerulosal cells at the surface of the lesion. The capsule has thickened considerably over the lesion,

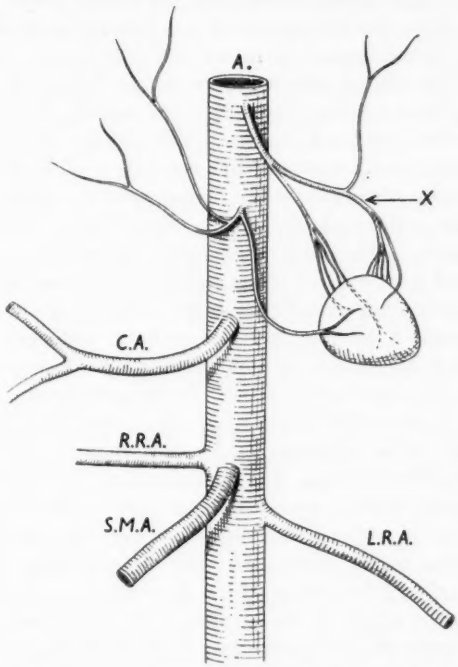
and from its deep surface fibrous tissue is extending down into the area of necrosis at its edges. The lesion itself has contracted considerably, being depressed from the surface and narrowed from side to side, taking on more the appearance of a thin wedge of fibrous tissue within the gland.

THE VASCULARIZATION OF THE ADRENAL IN THE RAT AND CAT

The finer branches of the adrenal arteries in the rabbit ramify on the surface of the capsule of the gland, and do not appear to anastomose with one another. This is also the case in the dog (Flint, 1900) and mouse and rat (Gersh & Grollman, 1941), in which the arteries in the capsule form only a poorly defined plexus. In the cat, however, Bennett & Kilham (1940) observed that the capsular arteries form a rich anastomosis, and that their arterioles are arranged in a freely anastomosing plexus. Since, therefore, there are differences in the degree of anastomosis of adrenal arteries in different animals, it was decided to determine the effect of interruption of single adrenal arteries in the rat and cat; the former having a vascularization similar to that in the rabbit, the latter having a more efficient anastomotic circulation. By this means it was hoped to estimate whether the results observed in the foregoing experiments on the rabbit adrenal are peculiar to this animal, and if so, whether this is a result of the manner of its vascularization.

The vascularization of the rat adrenal gland was determined in four animals by the method already described for the rabbit. A common arterial stem, taking origin from the anterior aspect of the aorta, is the main source of arterial supply of the gland (Text-fig. 2); this artery divides into two main branches which subdivide and supply the adrenal from its cephalic aspect. The more superior branch also gives off a twig to the inferior aspect of the diaphragm. Another small artery arises separately from the ventral aspect of the aorta to vascularize the medial aspect of the gland. No branches are given off from the renal artery to the adrenal on the left side, but on the right one or two such branches may be observed. In a female rat weighing 190 g., one of the two main branches reaching the cephalic aspect of the left adrenal was interrupted, and the animal was killed 3 days later. The resulting histological appearance of the gland is very striking (Pl. 2, fig. 4). There is an area of necrosis involving approximately one-third of the z. fasciculata of the cortex. The cells in this area of necrosis have lost their outlines, and their nuclei show varying degrees of karyolysis, but the network of fine argyrophil fibres remains. Over the surface of this necrotic zone the capillaries of the glomerulosal network and a thin outer rim of z. fasciculata are distended with erythrocytes. The cells of the z. glomerulosa here appear to be quite normal, but those in the outer rim of vascularized z. fasciculata are degenerate, like the cells in the remainder of the necrotic zone. The z. reticularis deep to the lesion is also quite normal, and its capillary plexus filled with erythrocytes. The remainder of the cortex shows very pronounced hyperaemia; cortical capillaries are so distended with erythrocytes that localized cystic dilatations are to be observed in the z. fasciculata. Most of these dilatations are small and restricted to the boundary zone between z. fasciculata and z. reticularis, but there are a few larger dilatations in the middle of the z. fasciculata.

The effect of interruption of an adrenal artery in the rat is, therefore, similar to that in the rabbit. Since fewer arteries vascularize the gland, however, division of a single artery in the rat produces a larger area of necrosis. In the case reported above, although one-third of the cortex shows necrosis, the medulla remains intact, thus demonstrating that the medulla in the rat adrenal is independent, to a large degree, of the blood reaching it through cortical capillaries. This is not surprising in view of the fact that the rat adrenal possesses arteriae medullae (Gersh &



Text-fig. 2. Diagram of the vascularization of the left adrenal of the rat. The aorta (*A.*) is shown giving origin to the coeliac artery (*C.A.*), right renal artery (*R.R.A.*), superior mesenteric artery (*S.M.A.*), and left renal artery (*L.R.A.*). The adrenal is seen to be supplied mainly by an artery which takes origin from the aorta and then divides into two branches, one of which gives off a twig to the inferior aspect of the diaphragm. The medial aspect of the gland is supplied by an independent artery from the aorta, which vascularizes the diaphragm principally. In the experiment described in the text an artery to the adrenal was interrupted at point *X*.

Grollman, 1941; Cain & Harrison, 1950). One important difference between the rabbit and rat adrenal cortex is that the capillary pattern can be determined in the latter by the erythrocytes remaining in the capillaries after death of the animal, a state of affairs used by Sjöstrand (1934, p. 85) to determine capillary activity in this gland.

The arterial supply of the cat adrenal differs markedly from that in the rabbit and rat in that a larger number of arteries (twenty or more) enter the gland at all points around its periphery from a variety of sources. Its richest supply appears to be from the renal artery, although branches come from the adrenolumbar,

coeliac, phrenic and, occasionally, the superior mesenteric artery, as well as the aorta, thus confirming the observations of Bennett & Kilham (1940). The arteries, particularly those from the renal artery, appear to anastomose quite freely on reaching the adrenal. Since the vascularization of the cat adrenal differs from that of the rabbit and rat, mainly in regard to the anastomoses between the arteries, it might be expected that interruption of individual adrenal arteries in this animal would not be effective in causing necrosis in the cortex. In one male cat, weighing 1·27 kg., in which a single artery arising from the coeliac artery to vascularize the cephalic pole of the gland was interrupted, it was found that an area of necrosis was produced, although its histological characteristics differed from those in the rabbit and rat. In the cat the adrenal cortex may be slightly lobulated, due to connective tissue septa dipping down into the cortex from the capsule. Interruption of a single artery, as just described, produced a necrosis of almost the whole of one of the small lobules present in the adrenal cortex of this cat 4 days after operation. The histological appearance is one of necrosis of the periphery of the lobule, having no regard for zonation as in the rabbit and rat. All the cells of the z. fasciculata, apart from a few cells in the centre of the lesion, show marked karyolysis, with disappearance of cell boundaries and complete loss of the regularity of arrangement of cells. A few glomerulosal cells persist over a small part of the surface of the lesion, but even these have lost their normal regularity and cell outlines, although their nuclei have retained some of their basophilia. A similar change is apparent in the cells of the z. reticularis in the deep aspect of the lesion. In the necrotic area of the lobule there is a dense network of argyrophil fibres, which also stains with Mallory's technique. The cells at the edge of an adjoining lobule show changes similar to those in the necrotic zone of the affected lobule. The medulla shows no degenerative changes.

Although the cat adrenal does not react in the same specific fashion as the adrenal of the rabbit and rat to arterial interruption, its arteries are just as much end-arteries to the adrenal cortex, particularly the z. fasciculata. It has been shown quite clearly that in the rabbit and rat the z. glomerulosa is much more resistant to interruption of a single artery to the adrenal than the z. fasciculata; this is presumably due to the more efficient anastomosis between capillaries of the glomerulosal network in these animals. In the cat the glomerulosal capillary network is probably just as efficient as in the rat and rabbit, over the greater part of the gland, but there does not appear to be any anastomosis between networks of adjacent lobules, when these are present. If the artery supplying one small lobule is interrupted, as in the above experiment, it would therefore be expected that necrosis of the whole lobule should follow, the necrotic process showing no discrimination between different zones.

DISCUSSION

Apart from the obvious importance of a knowledge of vascularization of the human adrenal gland for operations on the gland itself, it must be emphasized that the vascularization of the adrenal gland and kidney cannot be divorced from one another, as noted by Anson *et al.* (1947). Similarly, the gonadal artery may arise from a supernumerary renal artery or, even when the gonadal artery arises from the aorta, it may still pass through a hiatus in the renal vein. The ovarian artery

may even arise from an adrenal artery in 4% cases, according to Sauramo (1945), when the artery runs in close relation to the renal vessels. The possibility of division of the testicular or ovarian artery, or of the arterial supply to the adrenal from the renal artery, must therefore be considered in the operation of nephrectomy. The effect of division of the testicular artery before its anastomosis with the vasal and cremasteric arteries has already been discussed (Harrison, 1949), and only the possible effects of nephrectomy on the adrenal will be considered here.

The chief source of the adrenal arteries varies, being from the inferior phrenic artery (Pick & Anson, 1940) in some cases, from the renal artery in others. In the latter case the artery arising from the renal artery is usually single, and is considered by Gérard (1913) to be the principal artery supplying the adrenal, since it has the most numerous collaterals; it generally arises from the superior border of the renal artery near to the aorta, but it may arise in the neighbourhood of the kidney (twelve times in 209 adrenals (Gérard, 1913)). In such cases of late origin of the inferior adrenal artery it would be very difficult to avoid interrupting it during the operation of nephrectomy; but even an early origin of the inferior adrenal artery from the first part of the renal must be considered during this operation, since inadvertent inclusion of this artery in the renal pedicle could conceivably occur with short renal vessels. Since a renal origin of the inferior phrenic artery is present in 4·5% cases (Pick & Anson, 1940) the possibility of division of an adrenal artery during nephrectomy is a real one. It is therefore necessary to evaluate the consequence of this for the human adrenal.

The effect of division of a single adrenal artery in man would obviously depend on the functional capacity of its anastomoses with other arteries. Gérard (1913) has described the human adrenal as being surrounded by a complete arterial circle formed by the arteries supplying the gland; the arterial anastomoses which form this circle he describes as always being very slender, and only present in young subjects, never in adults. Anson *et al.* (1947) describe a striking arborization of adrenal arteries at the periphery of the gland, while Pick & Anson (1940), in depicting the arterial supply of the adrenal (their fig. 22), show no anastomoses between arteries from inferior phrenic, aortic and renal sources. It may be presumed, therefore, that the degree of anastomosis is similar to that existing in the cat, in which division of a single adrenal artery has already been noted to produce a localized area of necrosis within the gland. It would therefore be valuable to determine whether any derangement of adrenal cortical function is produced following a nephrectomy in man in which inadvertent division of an adrenal artery is known to have occurred.

One important observation resulting from the experiments described above is the susceptibility of the z. fasciculata, and the relative resistance of the z. glomerulosa and z. reticularis, to ischaemia resulting from division of an individual adrenal artery in the rabbit and rat. The z. glomerulosa is probably able to resist immediate damage by virtue of the more efficient anastomoses in its capillary plexus, but even this is insufficient to prevent subsequent damage to its cells at a later period following division of an artery. The blood supply of the three zones of the rabbit and rat adrenal, and their reaction to permanent ischaemia, thus reflects their relative independence morphologically and functionally, as already described by several authors (Deane & Greep, 1946; Harrison & Cain, 1947; Jones, 1949; Cain & Harrison, 1950).

SUMMARY

1. Attention is drawn to the manner of vascularization of the human adrenal gland, and the detailed arterial supply of the rabbit, rat and cat adrenal is described, confirming the work of Bennett & Kilham (1940) on the cat.

2. Individual adrenal arteries are end-arteries to the z. fasciculata of the rabbit adrenal cortex, as shown by the focal necrosis in this zone following immediately on their interruption. The z. glomerulosa also undergoes degenerative changes at a later period. If more than one artery to the rabbit adrenal is interrupted, the z. glomerulosa, as well as the z. fasciculata, tends to be included in the resulting area of immediate necrosis. Preliminary experiments suggest that individual adrenal arteries are also end-arteries to the adrenal cortex of the rat and cat, although the degenerative changes in the latter are not so selective as they are in the rat and rabbit.

3. The course of the histological changes in an area of necrosis in the rabbit adrenal cortex following interruption of individual adrenal arteries has been observed up to seven weeks after operation, when the glomerulosal cells degenerate.

4. The importance of these observations for the operation of nephrectomy is discussed, and it is shown that they confirm previous work on the relative morphological and functional independence of the zones of the adrenal cortex.

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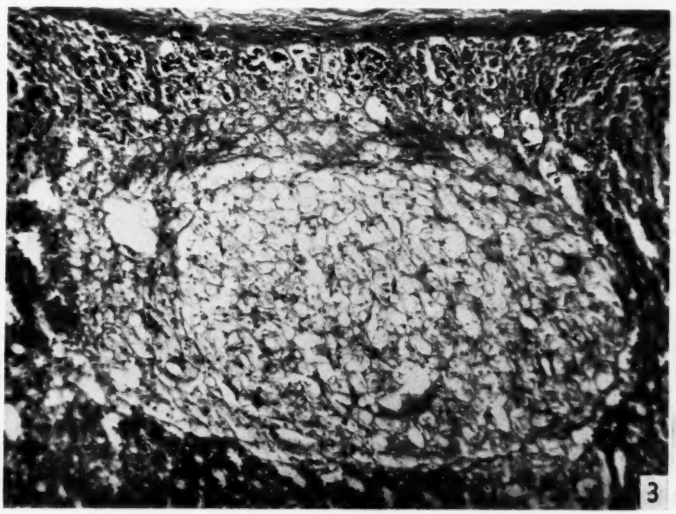

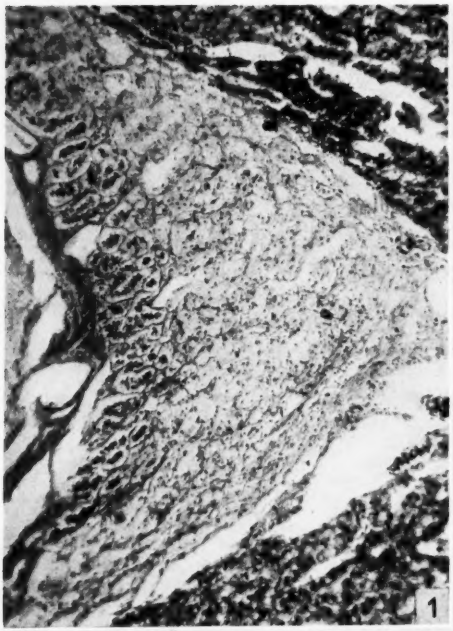
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EXPLANATION OF PLATES

PLATE 1

Fig. 1. An area of focal necrosis in the adrenal cortex of a rabbit 4 days after division of an artery arising from the adrenal branch of the adrenolumbar artery. The capsule is on the left of the figure; immediately under it is the z. glomerulosa which is not included in the lesion. The z. reticularis is also unaffected, but is not shown in the figure. The circumferential zone in which cells have completely necrosed, leaving only their reticular framework, is clearly shown. Note the complete disturbance of cellular pattern in the lesion, and the confluence of cells within it. (×95.)

Fig. 2. A portion of the adrenal cortex of a rabbit (rabbit 8) in which three arteries from the adrenal branch of the adrenolumbar artery, and one artery from the aorta, have been interrupted. The former has produced the large lesion, the latter the much smaller lesion to be seen in the upper part of the figure, removed from the z. glomerulosa. The larger lesion includes the z. glomerulosa, and has compressed the z. reticularis up against the medulla, the sinusoids of which can be seen on the right of the figure. Note also the distortion of the columns of the z. fasciculata due to the larger lesion in the lower part of the figure, and the density of this lesion due to the basophil particles contained therein. The circumferential zone can be seen around the larger lesion, particularly in the lower part of the figure. (×23.)

Fig. 3. The area of focal necrosis in a rabbit adrenal 2 weeks after interruption of an adrenal artery. The lesion can be seen to involve only the z. fasciculata. The capsule, with the subjacent z. glomerulosa, can be seen in the upper part of the figure. Vacuoles in the area of necrosis, due to degeneration of cortical cells, are clearly shown. Although the section from which this photomicrograph was prepared was stained with iron haematoxylin and eosin, the network of connective tissue fibres in the lesion is seen quite clearly. (×95.)

PLATE 2

Fig. 1. A photomicrograph of the area of focal necrosis produced in the rabbit adrenal cortex four weeks after interruption of an adrenal artery. The fragmented, thickened capsule is seen on the left of the figure. No normal z. fasciculata cells remain in the connective tissue matrix of the shrunken, triangular-shaped lesion itself. The z. glomerulosa cells over the surface of the lesion can still, however, be clearly distinguished. The spaces in the lesion in the lower part of the figure are due to dilated capillaries. Iron haematoxylin and eosin. (×78.)

Fig. 2. The condensed, fibrotic area produced in the adrenal cortex of a rabbit five weeks after division of an adrenal artery. The apex of the lesion reaches almost to the medulla, while the adjoining z. fasciculata cell columns have changed their orientation in relation to the lesion. The cells of z. glomerulosa can be seen to the left of the figure, enclosed in a dense capsular network of connective tissue. (×23.)

Fig. 3. A higher power view of the z. glomerulosa cells shown in the lower part of the last figure. These cells can be seen to have coalesced to form a syncytium. (×200.)

Fig. 4. Photomicrograph of the adrenal gland of a rat after division of an artery reaching the cephalic pole of the gland, which is uppermost in the figure. An area of necrosis has been produced in the z. fasciculata of the cortex. This contrasts markedly with the unaffected part of the cortex, which shows pronounced hyperaemia, the capillaries being distended with erythrocytes, and showing in this figure as darkly stained areas. The accumulation of erythrocytes can be seen to be mainly in the zone between z. fasciculata and z. reticularis where there are small cystic dilatations of capillaries; there are a few larger cysts in the middle of the z. fasciculata itself. The dark staining over the surface of the lesion is due to blood in the capillary network of the z. glomerulosa and a thin outer rim of z. fasciculata. The z. reticularis deep to the lesion is also seen to be unaffected. It must, therefore, receive a retrograde blood supply from the medulla. The medulla is quite normal. (×18.)

THE POST-NATAL DEVELOPMENT OF THE INFERIOR DENTAL NERVE OF THE CAT

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In most mammals the entire primary dentition is lost during the period of post-natal growth, in the cat between the ages of 7 and 9 months (Mivart, 1881). It has been shown previously (Mohiuddin, 1950) that just before and during this process, morphological changes indicating degeneration, can be demonstrated by silver impregnation methods in the nerve fibres within the pulp of the milk teeth. No changes of this character were seen in the stem fibres in the trunks from which the nerves of the teeth are derived, although it has been shown (Thomas, 1946) that more than half of the fibres in the inferior dental nerve are concerned with the innervation of the teeth and the gums.

So far, no quantitative observations have been made on the dental nerve trunks during the growth period, and it is possible that simple inspection might fail to reveal the loss of quite considerable numbers of fibres following the loss of the milk teeth. Whether this occurs or not, one might at least expect that the very marked change which occurs in the peripheral field of distribution would influence the pattern of post-natal development in the dental nerve trunks, and it was for the purpose of investigating this question that the present work was undertaken. The inferior dental nerve of the cat has been examined at various stages from birth to maturity in order to determine the number of axons, the proportion of myelinated axons, and the calibre spectrum of the myelin sheaths, at different ages.

MATERIAL AND METHODS

The inferior dental nerves of thirty-two healthy cats were examined. The ages of the animals are given in Tables 1 and 2. In the following description the term 'adult' refers to cats in which the entire primary dentition had been replaced by the secondary dentition, and the term 'young' refers to animals in which no deciduous teeth had been shed. One nerve was taken from each animal regardless of the side of the body, and in every case that part of the nerve lying between the point of origin of the mylohyoid branch and the mandibular foramen was examined.

Eighteen specimens were fixed and stained for the demonstration of myelin sheaths by the method described by Gutmann & Sanders (1943). This method is known to cause little shrinkage, probably not more than 10 %. To ensure complete penetration of the thicker nerves by the osmium tetroxide, it was necessary to prolong fixation for 3 days.

To demonstrate the axons, fourteen specimens which had been fixed *in situ* for more than 48 hr. by immersion in 10 % formalin in 0·85 % saline solution, were

* This work formed part of a Ph.D. Thesis accepted by the University of London in October 1949.

sectioned in paraffin at 7 μ and stained by Romanes' (1946) method. This method gave better results than several other silver techniques which were tried. A successful specimen shows no staining of connective tissue or nuclei of any kind, so that it is possible to distinguish very fine axons clearly and to avoid the mistake of counting nuclei as axons. Such specimens only were used for counting.

All the axons in each nerve were counted on photographs taken at a magnification of 400 diameters (Pl. 1, figs. 1–3), the method of Davenport & Ranson (1931) being used with slight modifications. Davenport & Ranson estimate a possible error of 10 % in their counts of axons in the spinal nerves of cats. The greatest single factor contributing to the error is the uncertainty of staining and microscopic resolution of the finest unmyelinated fibres. As there are relatively fewer unmyelinated fibres in the inferior dental nerve (except in the most immature specimens) than in spinal nerves, the error in the present series may be less than 10 %. For the same reason, the counts in the four newborn kittens (Table 1, S1–S4) are likely to be less accurate than in the other ten specimens, probably in the direction of an underestimation of the actual number of fibres present.

The total numbers of myelinated fibres were counted in photographs built up so as to cover the entire cross-section of each nerve in eighteen specimens. The photographs were made by projection directly on to bromide paper at magnifications of from 300 to 500 diameters according to the size of the nerve. The outer diameters of myelinated fibres were measured in random samples comprising over one-fifth of the total population in each nerve, so that about 1000 fibres were measured in each nerve. For this purpose separate photographs at a magnification of 700 diameters (Pl. 1, figs. 4–6) were used and the measurements were made by Causey's (1948) method. Each step of 1 mm. on the transparent scale was equivalent to 1·4 μ on the photographs. The total numbers of fibres measured in each specimen being different, it was necessary to convert the actual numbers in each fibre category into proportions of a thousand in order to obtain directly comparable figures.

OBSERVATIONS

The number of axons at different ages

The appearance of the axons in the inferior dental nerve of a newborn kitten is seen in Pl. 1, fig. 1. It will be noted that the axons are fine, and also that they are fairly uniform in size. In the adult cat most of the axons are much larger (Pl. 1, fig. 2), and there is considerable variation in size which is evidently the result of differential growth.

Axon populations at different stages are indicated in Table 1 and Text-fig. 1. The values range between the extremes of 4186 and 6993. The lowest figure was obtained in a 4-month-old specimen (S 7) in which, so far as could be judged from the silver preparations alone, there were very few unmyelinated fibres (Pl. 1, fig. 2). One older specimen (S 10) with a low population of axons had a similar appearance and a common feature of these two specimens was the relatively large size of most of the axons. These appearances were seen in several sections, prepared independently, from each specimen so that it does not seem likely that a failure in impregnation could account for the apparent lack of unmyelinated fibres. All the four newborn specimens had

fibre populations below 5000, but as explained earlier, these figures probably fall somewhat short of the true totals.

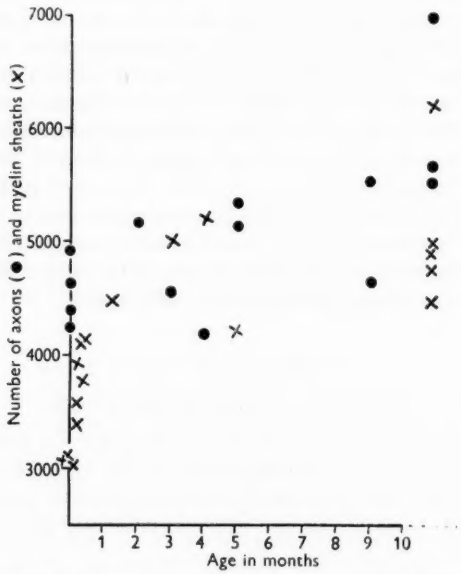
For the purposes of statistical analysis the fourteen specimens were subdivided into four different groups as follows:

(*a*) S1 to S4: in which no milk teeth had erupted; average number of fibres, 4548.

Table 1

Specimen number	Age	Total axons	Specimen number	Age	Total axons
S1	Newborn	4937	S8	5 months	5335
S2	Newborn	4382	S9	5 months	5132
S3	Newborn	4622	S10	9 months	4665
S4	Newborn	4252	S11	9 months	5533
S5	2 months	5167	S12	1 year or more	6993
S6	3 months	4555	S13	1 year or more	5527
S7	4 months	4186	S14	1 year or more	5677

The exact ages of specimens S1 to S7 are known. The ages of the others are approximations based on any information which could be obtained together with the general development and the state of the dentition.



Text-fig. 1. Graph showing the total population of axons and myelin sheaths at different ages. Each point on the graph represents a separate specimen.

(*b*) S5 to S9: in which all the milk teeth could be seen in a sound condition; average number of fibres, 4875.

(*a*+*b*) S1 to S9: in which no milk teeth had been lost; average number of fibres, 4730.

(*c*) S10 to S14: in which all the milk teeth had been replaced by sound permanent teeth; average number of fibres, 5679.

If group (*a*) is for the moment ignored, on the grounds that the counts on these specimens are probably the least accurate, and group (*b*), young animals, is compared with the adult group (*c*), there appears to be some increase in the number of fibres occurring during the period in which the milk teeth are lost and replaced by the permanent teeth, the average number of fibres present in each group being 4875 and 5679 respectively. On applying Fisher's '*t*' test (1948), however, a value for *P* of 0·1 is obtained, indicating a probability of 1:10 that the two groups come from a homogeneous population so far as the character in question is concerned. In these circumstances it is not possible to regard the apparent increase in number of fibres as significant.

If group (*a*), the newborn animals, is included with group (*b*), the average number of fibres in all animals younger than the age at which the milk teeth are lost becomes 4730, and comparing this with the average for adult animals (5679) and applying the '*t*' test, a value of between 0·01 and 0·02 is obtained for *P*. This does indicate a significant difference between the two populations, but it does not follow that there has been a real increase in the number of fibres present. It will be noted that the result is obtained only by the inclusion of the newborn specimens in which all fibres are very fine and where, for technical reasons, it is likely that the counts are an underestimate. It may well be that the statistical significance of the differences in the average counts of adult specimens on the one hand, and all young specimens on the other, is indicative of no more than the different technical difficulties of counting in the two groups, and it must therefore be concluded that no satisfactory evidence has been found for any increase in the number of fibres in the inferior dental nerve after birth, although it is possible that a small increase does occur between birth and the eruption of the milk teeth.

The number of myelinated fibres at different ages

The total populations of myelinated fibres are given in Table 2 and Text-fig. 1. The average in the three newborn specimens is 3028, and there is an increase during post-natal growth of 67 % to an average of 5051 in the adult cat. About one-half of the entire increase occurs during the first 10 days of life, which, for a sensory nerve, is a remarkably rapid rate of myelination. The population in the single 5-week-old specimen (W 10) was just within the adult range. Although it cannot be asserted that no further additions to the population of myelinated fibres can occur after 5 weeks, it can be seen from Text-fig. 1 that this process is probably completed by 2, and certainly by 3 months of age, and that no further change in the total number of myelinated fibres occurs when the milk teeth are lost.

The size of myelinated fibres at different ages

The appearance of the fibres in the newborn, the 3 months old, and the adult stages is shown in Pl. 1, figs. 4–6 respectively. As in the case of the axons, myelin sheaths in the newborn are small and of fairly uniform size (Pl. 1, fig. 4). The nerve in the adult (Pl. 1, fig. 6) clearly contains a proportion of small fibres, but most have grown larger to varying degrees. As will be seen, the nerve at birth is characterized by a unimodal distribution in fibre calibre, while in the adult distribution is bimodal.

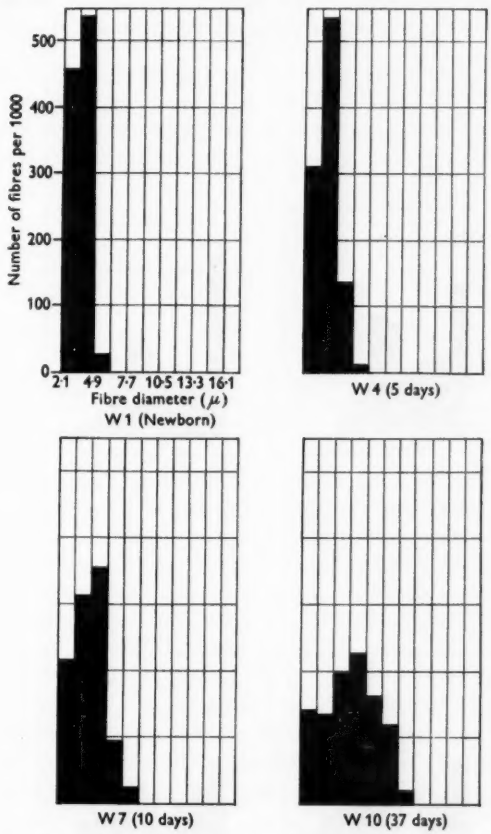
Table 2

Specimen number	Age	Total myelinated fibres	Range of fibre diameter in for each group											
			2·1–3·5	3·5–4·9	4·9–6·3	6·3–7·7	7·7–9·1	9·1–10·5	10·5–11·9	11·9–13·3	13·3–14·7	14·7–16·1	16·1–17·5	17·5–18·9
W1	Newborn	3030	433	540	27	—	—	—	—	—	—	—	—	—
W2	Newborn	3028	461	518	21	—	—	—	—	—	—	—	—	—
W3	Newborn	3027	451	520	29	—	—	—	—	—	—	—	—	—
W4	5 days	3937	310	539	138	13	—	—	–	—	—	—	—	—
W5	5 days	3588	315	519	150	16	—	—	—	—	—	—	—	—
W6	5 days	3395	316	505	162	17	—	—	—	—	—	—	—	—
W7	10 days	3775	216	312	355	93	24	—	—	—	—	—	—	—
W8	10 days	4105	216	390	330	55	9	—	—	—	—	—	—	—
W9	11 days	4118	245	398	327	27	3	—	—	—	—	—	—	—
W10	37 days	4489	141	136	196	226	161	120	20	—	—	—	—	—
W11	3 months	5016	229	128	118	100	148	161	74	40	2	—	—	—
W12	4 months	5202	278	81	101	91	124	159	102	38	20	6	—	—
W13	5 months	4271	158	106	96	45	109	136	121	90	80	54	5	—
W14	1 year or more	4904	195	80	101	65	65	145	107	72	99	53	18	—
W15	1 year or more	4903	180	116	68	48	57	85	113	97	106	75	38	17
W16	1 year or more	4487	156	123	100	71	112	135	104	80	73	41	5	—
W17	1 year or more	4760	146	107	70	46	77	136	137	107	102	66	6	—
W18	1 year or more	6202	130	117	94	73	113	197	97	94	86	41	8	—

N.B. The figures given for each fibre group are the proportions per 100 in the whole nerve, and not the actual number counted. The ages of specimens W1 to W11 were known exactly; the ages given for the other specimens are approximations based on any information which could be obtained, together with the general development and state of dentition.

This fundamental change in the character of the interior dental nerve first appears in the kitten aged 3 months (Pl. 1, fig. 5) and is present in all the older specimens.

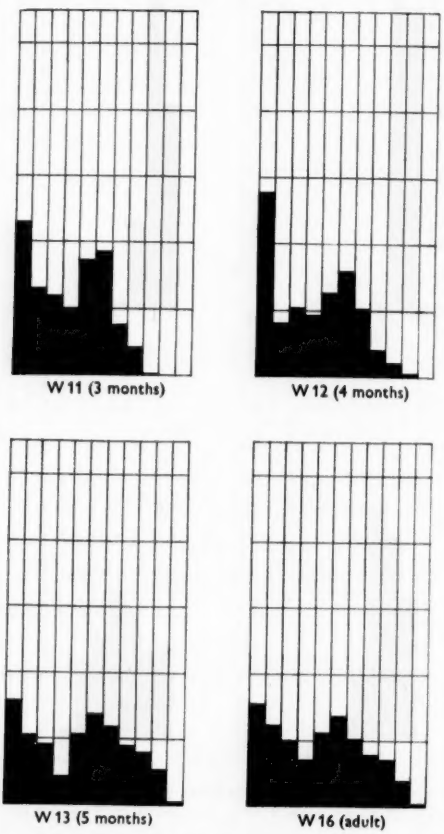
The results obtained by measurement are shown in Table 2, and histograms were constructed for each of these animals. Eight of these histograms are illustrated in Text-figs. 2 and 3, and have been selected to show all the phases in the development



Text-fig. 2. Histograms constructed to show the relative proportions of fibres in different size groups in the inferior dental nerve of specimens W 1, W 4, W 7 and W 10. The number of fibres in any size group per 1000 is given by the ordinate; the size groups are arranged along the abscissa and each has a range of 1·4μ. All the histograms in this figure and in Text-fig. 3 are to the same scale. For the detailed figures on which they are based see Table 2.

of the nerve. The histograms which have been omitted were identical in all essential features with those shown for similar stages of development. It can be seen that in the very immature specimens, W 1, W 4 and W 7 (Text-fig. 2), the fibre calibres are distributed around a single mode lying at about 4μ, but that a gradual shift to the right occurs during growth. Specimen W 10 (Text-fig. 2) shows an interesting transitional stage in which the shift to the right is so pronounced that the mode is now at

about 7μ. In this specimen there are relatively few fibres below 5μ and above 9μ in size. Histogram W11 (3-month-old kitten, Text-fig. 3) is bimodal and confirms the impression obtained from Pl. 1, fig. 5. The higher mode, lying at about 9μ, may have been formed by a continuation of the rightward shift seen in the younger specimens, and a new mode lying at about 4μ has been acquired, perhaps by the myelination of fibres previously unmyelinated. With further growth the lower mode remains stationary at 4μ, but the higher one comes to lie at about 10μ in the 5-month-old



Text-fig. 3. Histograms constructed to show the relative proportions of fibres in different size groups in the inferior dental nerve of specimens W 11, W 12, W 13 and W 16. For explanation, see Text-fig. 2.

specimen, W 13 (Text-fig. 3). The histogram of this specimen is very similar to that of the adult specimen W 16 (Text-fig. 3), indicating that the character of the spectrum is not affected by the loss of deciduous teeth which occurs after the age of 5 months. Growth in fibre size is complete at 5 months with a range of from 2 to $17{\cdot}5\mu$ distributed around two modes lying at about 4 and 10μ respectively. Only one adult specimen, W 15, possessed a few larger fibres.

DISCUSSION AND CONCLUSIONS

Few quantitative investigations of the dental nerves have been found in the literature. Nittono (1920) studied the growth pattern of the root and three main divisions of the trigeminal nerve in the rat, an animal which does not possess a deciduous dentition (Widdowson, 1948). He measured only ten of the largest fibres in each nerve bundle examined, but so far as they go his results indicated that increase in myelin sheath diameter was complete in about 3 months, which is rather earlier than in the cat's inferior dental nerve.

Windle (1926), Brashear (1936) and Thomas (1946) have studied the adult inferior dental nerve in the cat. Their results may be summarized as follows, after a minor adjustment of Brashear's figures to make them comparable with the others.

	Windle	Brashear	Thomas
Total axons	—	—	5642
Total myelin sheaths	—	—	5199
Percentage of unmyelinated fibres	17	22	7·9
Sizes of myelinated fibres (%)*			
Large	35	31	17
Medium	34	33	83
Small	31	36	

* Windle divided the myelinated fibres into 'large' (9μ and over), 'medium' (5–9μ), 'small' (less than 5μ); Brashear's division was into 10μ and over, 6–10μ, and less than 6μ in diameter. Thomas used only two divisions, 10μ and over, and less than 10μ in diameter.

It will be seen that Thomas's figures for total axons and myelin sheaths correspond closely with those obtained in adult specimens in the present investigation (5679 and 5051 respectively). His percentage of unmyelinated fibres is somewhat lower (7·9 against 11), but the difference is not great. Both Windle and Brashear obtained a much larger percentage, based, however, on counts of sample areas in the cross-section of the nerve and not on total counts. This may make their figures less reliable, but in any case, judging from the material examined in the present work, the proportion seems very variable. Reliable information on this point could be obtained only from total counts on a fairly large number of specimens in each of which both myelinated and unmyelinated fibres were counted. The present material suffers from the defect that myelinated fibres were counted in one set of specimens and total axons in another, so that only the averages of relatively small numbers of specimens can be compared.

When fibre size is considered, the proportions found in the present work for adult animals agree fairly well with Windle's results; his measurements were made directly on the sections, and it is generally recognized that this is not so satisfactory as the photographic method. This may account for the differences, namely 50 % of fibres more than 9μ in diameter in the present series and only 23 % between 5 and 9μ. Brashear's results are very similar to Windle's, but Thomas finds a much smaller proportion of large fibres and states that the majority lie between 4 and 7μ, a condition which suggests a unimodal distribution. It is possible that the histological technique used by Thomas, primary fixation in 5 % formalin, followed by decalcification before treatment with osmium tetroxide, has caused considerable shrinkage, thus distorting the quantitative results. Shrinkage was avoided by Windle and

Brashear and in the present work, by primary fixation with osmium tetroxide. This has been shown by Rexed (1944) to cause no appreciable change in fibre diameter. One may conclude that Thomas's figures for the total number of fibres and for the proportion of unmyelinated fibres in the adult nerve are confirmed by the present work, and that Windle's and Brashear's figures for the proportions of the different sizes of fibre, while approximately correct, give too small a proportion of large and too large a proportion of medium-sized fibres. Whether the condition is influenced in any way by the size, breed and sex of the animal has not been investigated.

When the growth pattern of the inferior dental nerve is considered it is clear from the results recorded that there is no obvious or direct relationship between any of the changes occurring in the nerve and the loss of the milk teeth and eruption of the permanent teeth. The adult proportion of myelinated fibres is acquired by the third month and the adult calibre spectrum by the fifth month. No satisfactory evidence for any post-natal change in the number of axons was found, though there is a possibility of a small increase occurring during the first 2 or 3 months. In other words, the inferior dental nerve acquires its adult characteristics while the milk teeth are functional and the permanent teeth developing but unerupted; no changes in the nerve trunk have been demonstrated which can be related to subsequent changes in the dentition.

When the growth pattern of the inferior dental nerve is compared with that of other nerves, although differences and similarities are found, these cannot be related in any specific way with the post-natal functional and developmental peculiarities of the dentition. On the whole, the similarities are more striking than the differences. For example, Hursh (1939) showed that the adult type of electrical responses could be elicited from both the saphenous and the cervical sympathetic nerves of the cat at about the third month. As there is an intimate relation between the sizes of the fibres and the type of electrical response, and the bimodal pattern is acquired by the inferior dental nerve at 3 months, it is probable that this nerve will have acquired its adult electrical characteristics at the same time as the two other nerves investigated by Hursh.

Many nerves are much less completely myelinated at birth than the inferior dental nerve of the cat. Boughton (1906) demonstrated a 175 % increase in the number of myelinated fibres in the oculomotor nerve of the same animal during the first 6 months of life. Studies of the spinal nerve roots by Hatai (1902), Dunn (1912) and Duncan (1934) in the rat and by Kjellgren (1944) in man show post-natal percentage increases in the number of myelinated fibres which vary very widely; from an insignificant or very small increase in the lumbo-sacral and cervical ventral roots in man (Kjellgren, 1944) to an increase of 135 % in the second ventral cervical root of the rat (Dunn, 1912) and 170 % in the posterior spinal nerve roots of the rat (Hatai, 1902). On the whole, there is a tendency for the dorsal roots to be less completely myelinated at birth than the ventral.

The post-natal increase in myelination in the inferior dental nerve is 67 %. Compared with other nerves, apart from some of the ventral spinal roots in man, this is small. It may be noted that all the myelinated fibres in the inferior dental nerve of the cat at birth are below 6μ in diameter. It has also been shown (Brashear, 1936) that the fibres in the pulp of the teeth are predominantly of similar calibre,

and at birth many of these fine fibres may already be mature, remaining in the same size group in the adult nerve. If so, their presence might be correlated with the early maturation of the milk dentition, and they might remain in the adult nerve to innervate the permanent teeth through collateral branches. This seems a likely interpretation of the findings, and would account for the relatively large number of fine myelinated fibres present in very immature nerves. At the same time the possibility has not been excluded that many or most of the fine myelinated fibres present in the adult nerve are derived from fibres unmyelinated at birth, and that the fine myelinated fibres present at birth have increased in calibre so as to enter the larger size groups (above 6μ) in the adult nerve.

One is forced to the general conclusion that while the proportion of myelinated fibres at any particular age may vary in different nerves in the same animal and in the same nerve in different animals, and while these variations may indicate different degrees of functional maturity, nevertheless, the loss of the milk teeth and maturation of the permanent teeth are not factors of importance so far as the developmental pattern of the dental nerve trunks is concerned. It appears also that adult electrical characteristics may appear in the cervical sympathetic and saphenous nerves of the cat (Hursh, 1939) at the same time as bimodality is first apparent in the inferior dental nerve. This suggests strongly that these three very different nerves all acquire adult electrical characteristics at about the same time, namely 3 months. Great importance cannot be attached to this in the absence of more extensive investigations of other nerves and in a number of different animals, but it is clear that any complete study of the development of a nerve must include a study of the development of the calibre spectrum. This may be more important than a simple assessment of the proportions of myelinated and unmyelinated fibres at different ages.

SUMMARY

1. An investigation of the development of the inferior dental nerve of the cat from birth to maturity was made in order to observe the reaction of the stem fibres in the nerve trunk to the changes in the field of terminal distribution resulting from the loss of the milk teeth.

2. The average number of axons in fully grown cats was found to be 5679. About 11 % of these are unmyelinated.

3. The number of axons does not increase during post-natal growth. It also remains unaffected by the loss of milk teeth.

4. The average number of myelin sheaths at birth is 3028. This increases by 67 % to 5051 in the fully grown animals.

5. The increase in the number of myelin sheaths occurs most rapidly during the first 10 days after birth, and it is probably completed by the end of the second month.

6. Loss of milk teeth does not affect the number of myelin sheaths.

7. The calibre spectrum of the myelinated fibres in fully grown cats is bimodal, one mode being at about 4μ and the other at about 10μ. The total range is from 2 to 17·5μ.

8. In the newborn kitten almost all the myelinated fibres are between 2 and 5 μ in diameter and the calibre spectrum is unimodal.

9. Growth in fibre size is gradual, and is completed in 5 months, although the bimodal character of the spectrum is evident by 3 months. The loss of the deciduous teeth does not affect the calibre spectrum.

I wish to thank Prof. F. Goldby for guidance at all stages of the work, and Dr J. J. Pritchard and Mr D. A. Scholl, for advice on statistical matters.

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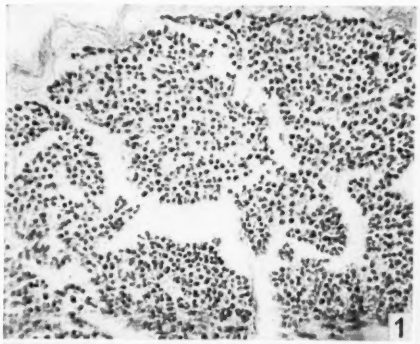
EXPLANATION OF PLATE

Figs. 1–3 are all transverse sections of the inferior dental nerve showing axons stained by Romanes' method; ×400.

Fig. 1. Newborn kitten. All the axons are fine and of nearly uniform size.

Fig. 2. Specimen S7 (4 months old). This specimen shows an unusually small number of fine unmyelinated axons.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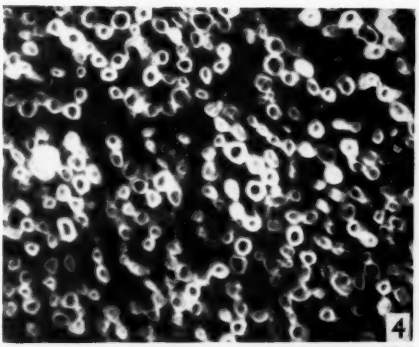
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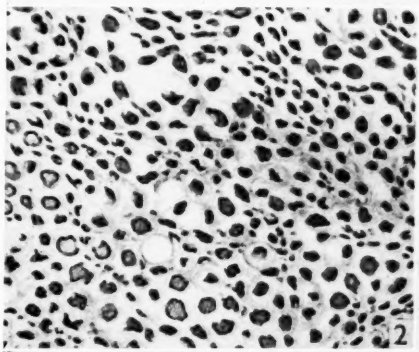
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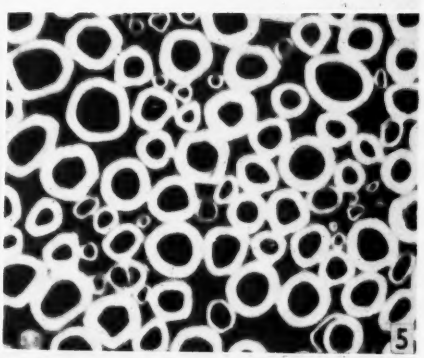
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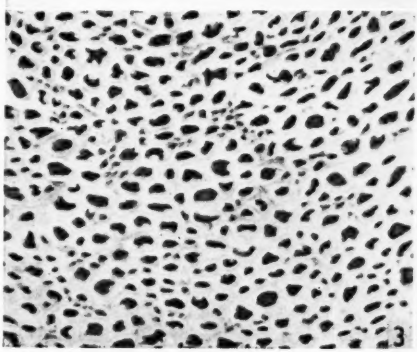
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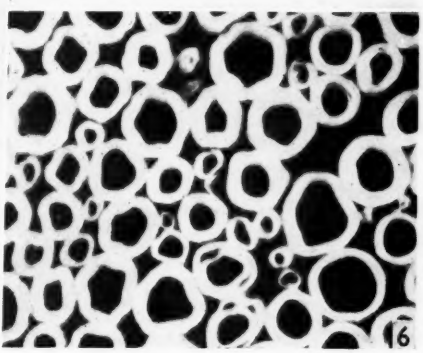
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Fig. 3. Adult cat. The axons vary greatly in calibre. Small groups of very fine axons, presumably unmyelinated, are a characteristic feature.

Figs. 4–6 are transverse sections of the inferior dental nerve stained by Kulschitzky's method to show myelin sheaths; × 700.

Fig. 4. Newborn kitten (W 1). All the fibres are fine and nearly equal in size.

Fig. 5. Three-month-old kitten (W 11). The fibres vary considerably in size and the histogram showed a bimodal calibre distribution.

Fig. 6. Adult cat (W 16). The prominence of large and small fibres clearly indicates the bimodal calibre distribution which was confirmed in the histogram.

THE DEVELOPMENT OF JOINTS CONCERNED WITH EARLY JAW MOVEMENTS IN THE SHEEP

By J. H. SCOTT, *Department of Anatomy, Queen's University, Belfast*

The late Sir Joseph Barcroft and his co-workers, during their investigation into the development of body movements in foetal sheep, noted that opening of the mouth occurred very early in gestation. They wrote (Barcroft & Barron, 1937): 'The embryo (42 days old and about 45 mm. C.R.) was executing rhythmic movements: they involved descent of the liver, opening of the mouth, flexion of the head and contraction of the infrahyoid muscles as in panting'; and (Barcroft, 1941): 'Within a week of the first reflex movement (33rd to 35th day) the movements in response to stimulus involve the mouth'; and again (Barcroft, 1941): 'Somewhere about the 70th day the movements of the mouth are very prominent. If an object be placed within the buccal cavity powerful rhythmic movements are often seen. These resemble chewing and sucking.'

The total gestation period for the sheep is 157 days (Barcroft, 1941). Simple opening and closing movements of the mouth, which are part of the primitive respiratory spasm, occur about the 40th day when the foetus is between 38 and 41 mm. long. It therefore seemed of interest to study the anatomy of the parts concerned in these early movements.

MATERIAL AND METHODS

The regions of the mandibular joint and of the middle ear were removed, embedded in paraffin and cut at 10 or 15 μ. Foetal sheep of 29–150 mm. C.R. length and of about 36–70 days' gestation were studied. The sections were stained by Mason's Ponceau-fuchsin-light green method or with Mallory's connective tissue stain.

RESULTS

A. The mandibular joint region

(1) 29 *mm. C.R. foetus* (*about the 37th day of gestation*)

A condensation of mesodermal tissue consisting of closely packed cells can be recognized as the primordia of the ramus of the mandible and its coronoid and condylar processes. The condensation for the head of the condyle turns medially over the upper surface of Meckel's cartilage so that the ramus and condyle together form an incomplete sleeve applied to the upper and outer surfaces, and in part to the lower surface of the cartilage. Into this 'sleeve' the masseter, and the medial and lateral pterygoid muscles are inserted. The condensation for the head of the condyle first develops at the place of insertion of the lateral pterygoid into the upper surface of Meckel's cartilage (Edgeworth, 1935); later the muscle appears to be inserted into the condylar condensation. This later condition has been reached in the 29 mm. foetus. The outer and upper surfaces of the condylar condensation are at this time continuous with the condensation for the zygomatic arch. There is as

yet no indication of any joint between the mesodermal condensation for the head of the condyle and the under surface of the zygoma; they are continuous with one another. Ossification has commenced in the body and ascending ramus of the mandible, and is extending into the base of the coronoid process but not into the condylar process. Ossification has also commenced at the back of the zygomatic arch in what will later become its posterior root. The coronoid process is continued backwards into an intramuscular fibrous septum within the substance of the temporal muscle. The coronoid process grows as a result of a backward spread of the process of ossification into this septum.

(2) 38 *mm. c.r. foetus* (*about the 39th day*)

The ramus of the mandible, with the coronoid process and the condyle, are differentiated as parts of a dense mesodermal condensation which lies above and on the outer side of, and also in contact with Meckel's cartilage below and behind the orbit. Ossification extends from the ramus into the root of the coronoid process and to a slight extent into the neck of the condyle; the bulk of the condylar head is still a mass of dense cellular tissue in which there is neither cartilage nor bone. To the lateral side of, and above, the condylar condensation there is the developing zygomatic arch which, lateral to and behind the coronoid process, sends its anterior root inwards towards the side of the cartilaginous cranial wall above the condensation for the head of the condyle. Ossification of the zygomatic arch extends forwards from its posterior root, which lies to the lateral side of the middle ear, as far as the developing zygomatic bone beneath the orbit. As yet the process of ossification has not extended into the anterior root which will later form the roof of the temporo-mandibular joint. Between the mesodermal condensations of the head of the condyle and of the developing anterior root of the zygoma the mesenchyme has become less dense. It is here that the mandibular joint at a later stage will develop. At this time, however, there is no indication of any joint cavity. The outermost portion of the mesenchymal tissue between the anterior root of the zygoma and the head of the condyle is made up of a more dense cellular tissue so that the condensations for the head of the condyle and the zygoma appear to be continuous. This region marks the site of the lateral part of the joint capsule and the temporo-mandibular ligament.

(3) 41 *mm. c.r. foetus* (*about the 40th day*)

The stage of development of this region is similar to that seen in the 38 mm. foetus.

(4) 48 *mm. c.r. foetus* (*about the 43rd day*)

The anterior root of the zygoma is now a well-differentiated mesodermal condensation above the developing head of the condyle. Between the root of the zygoma and the head of the condyle there is a zone of highly vascular, loose tissue, the synovial mesenchyme, in which, at a later stage, the joint cavities will commence to form. Apart from an increase in size of the various parts, there has not been much change in the relations of the parts as described for the 38 mm. foetus.

Muscles of mastication

At this time, according to the experimental work of Barcroft, opening and closing movements of the mouth can be produced by stimulation of the foetus. These movements must take place at the joint between the malleus (with which Meckel's cartilage is continuous) and the incus, as the temporo-mandibular joint has not yet developed. At this time the attachments of the muscles of mastication are as follows:

Masseter. Arises from the zygomatic arch and is inserted into the outer surface of the mandibular ramus, both of which parts have at this time a core of growing bone.

Temporal. Arises from the cartilage forming the side wall of the cranium above the otic capsule and from the developing bone of the squamous part of the temporal. It passes forwards to be inserted into the coronoid process into which ossification has now extended from the ramus of the mandible.

Medial pterygoid. Arises in part from the under surface of the processus alaris (ala temporalis) of the sphenoid and in part from the developing vertical plate of the palatine bone. It is inserted in part into the perichondrium of the inner surface of Meckel's cartilage (its original insertion) and in part into the lower border of the ramus of the mandible.

Lateral pterygoid. Arises from the under surface of the processus alaris and is inserted into the head of the condyle which has, as yet, no cartilaginous or osseous basis.

Tensor tympani. Arises from the under surface of the processus alaris and runs backwards and downwards to become attached to the descending process of the malleus. It is at this time a muscle of mastication.

Mylohyoid. The mandibular attachment of this muscle is in part to the perichondrium of the inner side of Meckel's cartilage and in part to the developing body of the mandible.

It will be noticed that at this time the more important muscles are in the process of gaining attachment to bony structures and loosing their attachment to cartilaginous structures. This is true of the masseter, temporal, medial pterygoid and mylohyoid muscles.

(5) 50 *mm. c.r. foetus* (*about the 44th day*)

As in the 48 mm. foetus (Pl. 1, fig. 2).

(6) 55 *mm. c.r. foetus* (*about the 46th day*)

The mesenchyme between the head of the condyle and the anterior root of the zygoma now forms a disc of denser tissue, the joint disc. The cells of the disc are orientated so as to lie parallel with those of the articular surface of the head of the condyle. There is, however, a slight loosening of the tissues over the upper surface of the disc, between it and the under surface of the condensation for the anterior root of the zygoma. The central portion of the head of the condyle is composed at this stage of pre-cartilage. Ossification is extending into the anterior root of the zygoma behind the head of the condyle.

(7) 60 *mm. c.r. foetus* (*about the 47th day*)

The dense tissue of the joint disc between the head of the condyle and the anterior root of the zygoma is well differentiated. The disc is continuous on the outer side with the capsule of the developing joint and the developing temporo-mandibular ligament. On the inner side some of the fibres of the lateral pterygoid muscle are attached to the disc as well as to the head of the condyle. At the periphery of the joint region, especially towards the front and outer sides, the mesenchyme between the joint disc and the zygoma is quite loose, and this loosening of the mesenchyme is the first indication of the development of the upper synovial cavity. The head of the condyle consists of a core of hyaline cartilage surrounded by a thick, dense zone of mesodermal cells. The cartilage is being replaced by bone from the neck of the condyle and is continuing to grow by a conversion of the sub-articular mesodermal cellular zone into cartilage. Ossification now extends from the zygomatic arch into the anterior root of the zygoma towards the side of the cranium above the head of the condyle. In this manner a bony roof is formed for the developing joint. In development this roof extends from behind forwards. It would appear that this extension of the process of ossification into the anterior root of the zygoma and the development of the joint disc are associated with each other. Until the roof of the mandibular fossa ossifies the tissue over the growing condyle is loose mesenchyme, except where the outer side of the condyle is continuous with the zygomatic arch. After ossification has spread into the anterior root of the zygoma the growing condyle will compress the intervening tissue against the now rigid roof of the mandibular fossa and so produce the joint disc which at a later date will become the definitive articular disc. The appearance of the articular disc and of the upper and lower compartments of the temporo-mandibular joint seems to be associated with a falling off of the rate of growth of the head of the condyle relative to the adjacent parts and a reduction of the pressure exerted between the head of the condyle and the roof of the mandibular fossa.

(8) 64 *mm. c.r. foetus* (*about the 48th day*)

The joint disc is now separated over the greater part of its upper surface from the root of the zygoma by a zone of very loose mesenchyme, in which the synovial cavity of the upper compartment of the joint is in the process of formation by a separation of the mesenchymal cells. These cells form an irregular lining to the developing cavity. Towards the front of the joint area and to its outer side a similar process is commencing to separate the dense tissue of the joint disc from the articular surface of the head of the condyle. In this way there is formed the definitive articular disc which will separate the two synovial cavities of the functional joint.

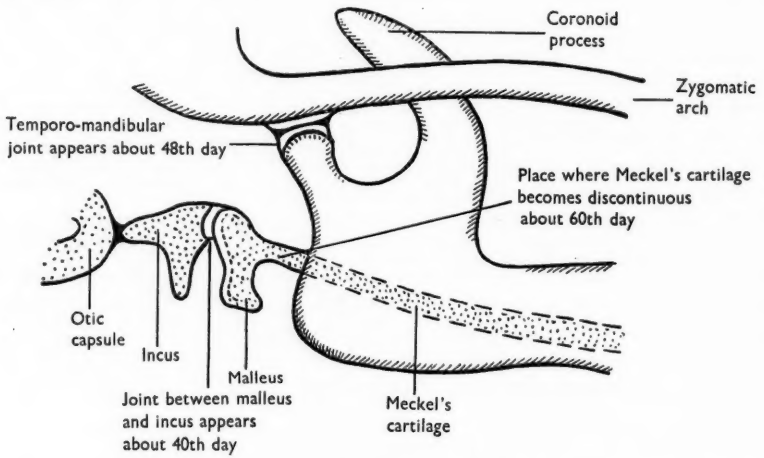
(9) 72 *mm. c.r. foetus* (*about the 50th day*)

Joint cavities are now developing above and below the joint disc (articular disc); the upper cavity, which was the first to appear, is more extensive than the lower. Loose tags of mesenchymal tissue project into the joint cavities. Towards the periphery the cavities are extending by a loosening of the mesenchyme (Pl. 1, fig. 3). A similar condition exists in a 68 mm. foetus, so that it can be said that both synovial

cavities of the mandibular joint have commenced to develop about the 48th–49th day of gestation. The centre of the head of the condyle is composed of hyaline cartilage which is being replaced from below and at the sides by bone. The cartilage is separated from the lower compartment of the joint by a thick zone of densely packed mesodermal cellular tissue into which the cartilage is extending, and by a superficial layer of developing fibrous tissue which forms the articular surface of the head of the condyle.

(10) 125 *mm. c.r. foetus* (*about the 60th day*)

The articular disc and the upper and lower compartments of the joint are now well differentiated, and the head of the condyle is growing in all dimensions by a proliferation of its cartilage.



Text-Fig. 1. Diagram to show the parts concerned in the development of the temporo-mandibular joint and the joint between the malleus and incus.

B. The middle ear region

(1) 29 *mm. c.r. foetus* (*about 37th day of gestation*)

The malleus, incus and stapes are all represented in cartilage. The posterior process of the incus is attached to the cartilage of the otic capsule by a dense disc of mesodermal tissue, the 'joint disc' (Haines, 1947), but there is no evidence of any joint cavity. The malleus shows two processes, the handle which descends in loose mesenchyme between the canal of the outer ear and the pharyngo-tympanic recess, and an anterior process which is continued into Meckel's cartilage. The cartilages of the malleus and incus are connected by a plate of dense mesoderm, but there is as yet no joint cavity between them (Pl. 1, fig. 1). The tensor tympani muscle is attached to the medial side of the handle of the malleus and the stapedius to the posterior surface of cartilage of the stapes.

(2) 34 *mm. c.r. foetus* (*about the 38th day*)

The dense plate of mesodermal tissue (the joint disc) which connected together the malleus and the incus in the 29 mm. foetus has become much looser in texture, and the first indication of the synovial cavity of the developing joint is seen in the separation of the cells of the disc to form an irregular space between the adjacent (articular) surfaces of the cartilages. At the periphery of the disc tissue the mesodermal cells form a primitive capsule for the developing joint, continuous with the perichondrium of the cartilages. The condition is similar to that described by Haines (1947) in the early development of limb joints.

(3) 41 *mm. c.r. foetus* (*about the 40th day*)

There is still no sign of a joint cavity between the incus and the side of the otic capsule nor between the incus and the stapes. Between the incus and the malleus a synovial cavity is forming, but the outline of the cavity is still irregular and it is crossed by some strands of mesenchymal tissue. At the periphery of the joint disc, in which the synovial cavity is being formed, the mesenchyme forms a well-differentiated capsular ligament which limits the extent of the developing synovial cavity.

(4) 50 *mm. c.r. foetus* (*about the 44th day*)

The joint between the malleus and incus is now well formed; the cells of the joint disc which line the synovial cavity still form irregular strands projecting into or crossing the cavity, and everywhere this tissue separates the cavity from the articular surfaces of the cartilages. At the periphery of the joint the developing capsule is well formed (Pl. 1, fig. 2).

(5) 55 *mm. c.r. foetus* (*about the 46th day*)

The joint between the malleus and incus continues to extend as the articular areas of contact between the two cartilages increase with growth. In the central part of the joint the cavity is now lined by a single layer of flattened cells lying on the surface of the cartilages; towards the periphery, where the joint cavity is extending, the mesenchymal cellular layer is thicker and more irregular.

(6) 60 *mm. c.r. foetus* (*about the 47th day*)

There is not much change from the condition described in the 55 mm. foetus. Meckel's cartilage is still a continuous bar stretching from the malleus to the mental symphysis.

(7) 72 *mm. c.r. foetus* (*about the 50th day*)

The joint between the malleus and incus is extensive and well developed, but there is still no evidence of any joint cavity between the incus and stapes or between the incus and the otic capsule. The external auditory canal consists of a tube filled by closely packed epithelial cells (Pl. 1, fig. 3).

(8) 125 *mm. c.r. foetus* (*about the* 60*th day*)

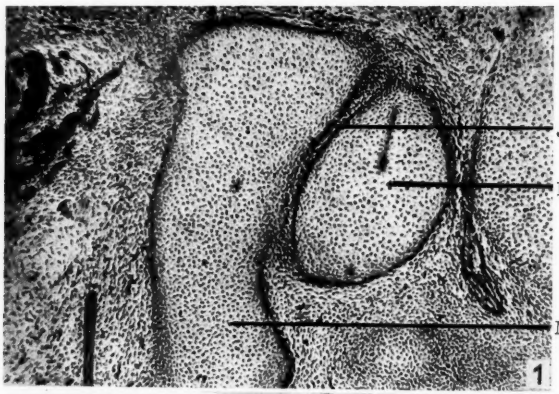
The joint between the malleus and incus may show the secondary 'fusions' between the articular surfaces of the cartilages which Haines (1947) describes as artefacts. There is still no evidence of any other joint cavity between the middle ear ossicles. Ossification has commenced in the anterior process of the malleus where it passes through the petro-tympanic fissure, and the continuity between the malleus and Meckel's cartilage is commencing to dissolve just proximal to this area of ossification. Meckel's cartilage has now a free distal end which is attached to the ossifying tip of the anterior process of the malleus by a cord of fibrous tissue. This cord lies just to the inner side of the head of the condyle and the temporo-mandibular joint. It is at about this time that calcification commences in the dentine of the cheek teeth.

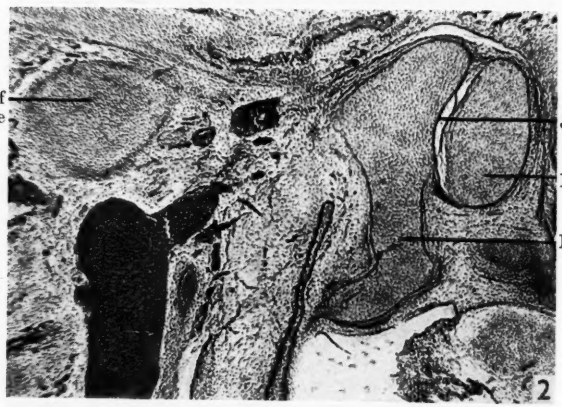
(9) 150 *mm. c.r. foetus* (*about the* 70*th day*)

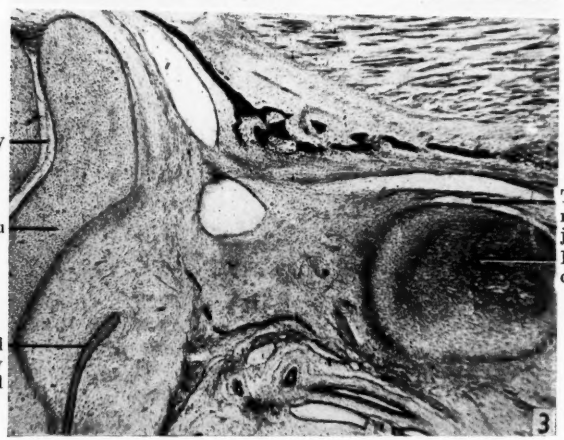
Ossification has commenced in the head of the malleus, and extends from here into the roots of the handle and of the anterior process. The pre-ossification stage of vascular tissue invasion is seen involving the head of the incus. The degeneration of the distal end of Meckel's cartilage has gone further than at the 125 mm. stage, the remains of the cartilage being shown by a fibrous tissue cord which passes from the base of the skull to the lower part of the inner surface of the mandibular ramus. There is still no evidence of any of the other middle ear joint cavities.

DISCUSSION

The purpose of this investigation was to study the anatomy of the parts concerned in the jaw movements during foetal life. Foetal sheep were used because Sir Joseph Barcroft and his co-workers had already correlated foetal age and foetal measurements, and had also noted the time when jaw movements commenced. As a result of this present study, it would seem that jaw movements first appear soon after the development of the joint between the future malleus, which is at this early stage in continuity with Meckel's cartilage, and the incus. There is as yet no temporo-mandibular joint. When the temporo-mandibular joint begins to develop about a week later jaw movements will involve two joints on each side of the face and this state of affairs continues for some two weeks before the continuity of Meckel's cartilage becomes broken, and jaw movements will henceforward involve only the temporo-mandibular joints. During the period of foetal life when both the temporo-mandibular and the incudo-malleolar joints are acting together the movements of the lower jaw can only be simple in nature and limited in amount. As increasing complexity of jaw movements appears to be related to greater differentiation of the nervous system, it is probable that the breaking of the continuity of Meckel's cartilage which takes place where it passes to the inner side of the temporo-mandibular joint is the result of the stresses falling upon this portion of the cartilage as the later joint develops and the more complex movements involved in chewing begin to appear in the foetus. While the foetal jaw movements are being transferred from the incudo-malleolar to the temporo-mandibular joint there is at the same time a migration of those muscles which were attached to Meckel's cartilage to their definitive mandibular







SCOTT—DEVELOPMENT OF JAW JOINTS IN THE SHEEP

attachments. It is interesting to notice that it is that segment of Meckel's cartilage to which the pterygoid muscles and the mylohyoid were attached which atrophies. The tensor tympani retains its attachment to the malleus, and the only other portion of the cartilage which remains for any length of time is at the mandibular symphysis where it is still present in a 250 mm. foetal sheep.

SUMMARY

1. The temporo-mandibular and middle ear regions of foetal sheep from 29 to 150 mm. in length (36–70 days gestation) have been examined in serial sections.

2. Up to about the 37th day (30 mm.) there is no joint cavity between the malleus and the incus and there is no evidence of any opening or closing of the mouth.

3. Between the 38th and the 40th day (34–38 mm.) the joint cavity between the malleus and incus commences to form, but there is no evidence whatever of a mandibular joint. The muscles of mastication include the tensor tympani of the middle ear. This is the time at which Barcroft has shown that opening and closing of the jaws begins.

4. The first indication of a joint cavity and articular disc at the mandibular joint is found about the 47th day of gestation when the foetus is about 60 mm. in C.R. length, and these structures are well formed by the 50th day. At this time, however, Meckel's cartilage is still a continuous bar stretching from the malleus to the mental symphysis, so that jaw movements involve two joints, the incudo-malleolar and mandibular joints.

5. Meckel's cartilage commences to break its continuity with the anterior process of the malleus to the inner side of the mandibular joint at about the 60th day of gestation (C.R. length 125 mm.). Calcification commences in the crowns of the teeth at the same time, and ossification of the ear ossicle commences soon after (in the 150 mm. foetus).

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EXPLANATION OF PLATE

Fig. 1. 29 mm. foetal sheep (about 37th day of gestation). The back portion of Meckel's cartilage (the malleus) is separated from the incus by a 'joint disc'. At this stage there are no joint movements.

Fig. 2. 50 mm. foetal sheep (about 44th day of gestation). The joint cavity between the back of Meckel's cartilage (the malleus) and the incus is forming. Jaw movements take place at this joint. The temporo-mandibular joint has not yet developed.

Fig. 3. 72 mm. foetal sheep (about 50th day of gestation). The temporo-mandibular joint has developed and jaw movements involve two joints on each side of the head, as Meckel's cartilage is still continuous with the malleus.

THE HISTOLOGY OF THE HUMAN FOETAL AND PREPUBERTAL PROSTATES*

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Histological changes that have been described in the prostates of the human foetus and newborn child are squamous metaplasia (Schlacta, 1904; Stieve, 1930; Peter, 1938; Brody & Goldman, 1940) and a focal hyperplasia of fully differentiated epithelium (Schlacta, 1904; Moore, 1936; Brody & Goldman, 1940). Moore (1936) found this focal hyperplasia in the prostates of about 10 % of full-term infants under 7 days old, but it was not present in the prostates of premature infants. These changes appear to be sufficiently variable to warrant further study.

Moore & McLellan (1938) pointed out the similarity between these changes in the immature prostate and those occurring in benign hypertrophy. According to Lowsley (1930) the prostate can be divided into five lobes. Two lateral lobes are situated one on each side of the urethra and extend anteriorly and posteriorly; their ducts pass posteriorly, then medially and anteriorly to end in the lateral furrows of the urethra. A middle lobe is situated behind the upper part of the urethra and is bounded above by the bladder and below by the ejaculatory ducts; its ducts open into the posterior wall of the urethra above the ejaculatory ducts. A small anterior lobe is situated in the median plane anterior to the upper part of the urethra, and its ducts open into the anterior wall of the urethra. The fifth or posterior lobe is bounded anteriorly by the urethra, superiorly by the ejaculatory ducts and posteriorly by the capsule; its ducts end in the posterior wall of the urethra below the ejaculatory ducts. Lowsley considers that a distinct layer of fibrous tissue separates the posterior and lateral lobes. Benign hypertrophy commonly affects the lateral and middle lobes but seldom involves the posterior lobe (Moore, 1943), whereas the posterior lobe is regarded as the common site of carcinoma (Scholl, Hinman, von Lichtenberg, Hepler, Gutierrez, Thompson, Cook, Wildbolz & O'Conor, 1945). It is obvious, therefore, that the distribution of the changes in the prostates of the foetus and newborn child is of great importance if any connexion is to be established between them and pathological changes occurring in later life.

Carcinoma of the prostate may sometimes be preceded by a specific type of hyperplasia which is usually found in prostates with benign hypertrophy, but is also seen occasionally in normal prostates (Andrews, 1949). An attempt was made to discover at what age this hyperplasia first appears and to see whether or not it formed part of the changes occurring at birth.

The role of the milk factor in the production of carcinoma of the breast in mice is well known, and it is perhaps conceivable that similar factors may act in the production of human carcinoma. Since the presence of the milk factor in the breasts of young mice may be determined histologically (Pullinger, 1947), the relationship

* This work formed part of a dissertation approved for the degree of Doctor of Medicine in the University of Bristol.

between the histological changes in the immature prostate and breast feeding was also studied.

MATERIAL AND METHODS

The material consisted of sixty-six prostates which were removed at autopsy from infants and children between the twenty-fourth week of foetal life and 13 years of age. The prostates were fixed whole in 10 % formol saline (1 part commercial formaldehyde with 9 parts normal saline) for 4 days, the solution being changed on the second day. The majority were then completely divided into blocks 1–2 mm. in thickness by making cuts horizontally at right angles to the urethra. Some of the larger ones were sectioned sagittally, but in every case the whole prostate was completely divided into blocks which were embedded in paraffin and sectioned at 4 μ.

The sections were stained with Weigert's haematoxylin and 1 % alcoholic eosin. Representative sections at various ages were also stained with French's elastic stain (1929).

For the purpose of study the material was divided into groups.

Group 1: nineteen premature infants who survived from 5 hr. to 15 days after birth.

Group 2: six stillborn full-term infants.

Group 3: sixteen full-term infants under the age of 7 days.

Group 4: six infants between the ages of 7 days and 1 month.

Group 5: ten infants between the ages of 1 and 9 months.

Group 6: nine children between the ages of 9 months and 13 years.

With the exception of groups 2 and 6 each group was then subdivided into breast-fed, not breast-fed, and those with no reliable history of feeding. In these four groups the totals were: breast-fed, 21; not breast-fed, 17; doubtful, 13.

HISTOLOGY

The prostate of the 24-week foetus, the youngest examined, showed few tubules, separated widely by supporting stroma (Pl. 1, fig. 1). They were surrounded by a thin layer of hyaline material and lay in a stroma of loose cellular connective tissue which separated them from the main supporting stroma and smooth muscle of the gland. The tubules were mainly composed of solid collections of small cells with small pyknotic nuclei. The cells were polygonal in shape with indistinct, scanty cytoplasm and their nuclei were irregular except at the periphery where they were thin, elongated and arranged parallel to one another in palisade form. Some of the tubules had a lumen and the lining cells were usually cubical with deeply eosinophil, well defined cytoplasm and thin pyknotic nuclei usually arranged perpendicular to the lumen, but some cells were flatter and their nuclei irregular (Pl. 2, fig. 4). A small amount of eosinophil secretion was sometimes present in the lumen. A few of these tubules with lumina were composed of a single layer of lining cells and a basal layer of epithelial cells arranged horizontally.

Some of the luminal cells, and a few of those at the centre of the solid tubules, had larger nuclei, which were round or oval in shape, pale-staining, with a delicate chromatin network and sometimes a nucleolus. This nuclear change was often accompanied by an increase in the size of the cell and perinuclear vacuolation of the cytoplasm.

From the 24th week of foetal life until term there was a considerable increase in the size of the prostate, due mainly to a proliferation of the glandular epithelium, so that at term the prostatic tubules, the majority of which were still surrounded by a thin layer of hyaline material, lay closer together, and those with lumina predominated (Pl. 1, fig. 2). During this period there was also an alteration in the histological appearance of the cells. The large, round or oval, pale-staining nuclei increased in number and eventually predominated. Vacuolation of the cytoplasm, usually perinuclear but occasionally subnuclear, was common and was accompanied by a clearing of the cytoplasm and secretion of eosinophil granules into the lumen. This change began in the luminal or central cells and was progressive until all the cells were involved with the exception of the basal layer of cells which usually retained their narrow pyknotic nuclei and made a striking contrast with the luminal cells (Pl. 2, fig. 5). In some glands, however, even these basal cells were altered.

The nuclear changes in the majority of tubules were accompanied by an increase in the size of the cell, so that at term the tubules were lined by low columnar cells with well-defined cell borders and a clear reticular cytoplasm except for a thin rim of eosinophil material at the luminal border (Pl. 2, fig. 6). In others, however, although the cells showed nuclear and cytoplasmic changes, they did not increase in size and the tubules were lined by a flatter type of epithelium (Pl. 3, fig. 8).

It was interesting that at this stage some of the tubules did not possess a basal layer of epithelial cells but were lined by one layer of cells only (Pl. 3, fig. 9).

Table 1. *Incidence of the histological changes seen in the foetal and prepubertal prostates*

(The degrees of epithelial proliferation and squamous metaplasia are recorded in increasing values of +.)

Age groups	Total no. of prostates	Incidence and degree of proliferation of the tubule epithelium					Incidence and degree of squamous metaplasia					Prostates with cyst formation	Prostates with hyperplasia and dilation of ejaculatory ducts	Prostates with adult epithelium
		−	+	++	+++	++++	−	+	++	+++	++++			
1 (premature)	19	0	5	7	3	4	0	8	7	3	1	6	6	7
2 (stillborn)	6	0	0	1	4	1	0	0	3	3	0	3	4	5
3 (under 7 days)	16	0	1	6	9	0	1	1	5	7	2	8	10	9
4 (7 days–1 mth.)	6	0	0	2	4	0	1	3	1	0	1	2	5	5
5 (1–9 mths.)	10	3	4	1	2	0	10	0	0	0	0	3	8	6
6 (9 mths.–13 yr.)	9	7	2	0	0	0	9	0	0	0	0	0	1	5

The majority of prostates in groups 1 and 2 showed this proliferation and alteration of the tubule epithelium. These changes were present equally in all lobes and, in general, gradually increased with maturity up to term (Table 1). In some prostates, however, both the proliferation and the alteration of the epithelium were very marked, and in one gland the proliferation had occurred focally, producing a small nodule. In a few prostates the changes were only slight and the histological appearances more closely resembled those of the 24-week foetus than other prostates of the same age period.

From birth until the end of the first month of life there was little change in the

histological appearance, but between the first and fourth month there were gradual regressive changes in the epithelium. The tubules near the urethra were the first to acquire a characteristic appearance. They were lined by stratified columnar epithelium with the exception of the beginning of the tubules arising from the upper part of the urethra which were lined by transitional epithelium. In the stratified columnar epithelium the luminal cells presented a characteristic appearance, each having a distinctive eosinophil cytoplasm and a small dark oval nucleus lying parallel to the long axis of the cell; the palisade appearance thus produced was quite striking. These tubules were usually surrounded by a wide ring of connective tissue. Farther away from the urethra the number of cell layers lining the tubules was gradually reduced until there were only a luminal and a basal layer. The height of the luminal cells was also reduced so that they became cubical or, occasionally, even flattened.

The terminal parts of the tubules varied. Relatively few solid collections of cells were present and the tubules with a lumen were lined by one or more layers of small cells, usually cubical, but often irregular and sometimes flattened. In general, after the fourth month, the terminal tubules were similar in appearance to those of the 24-week foetus (Pl. 2, fig. 7).

The regression of the epithelium was not always complete; some cells retained the larger pale-staining nuclei but even these cells were smaller than the average cell at term.

From the fourth month until the age of 13 years the only change was a slight increase in the size of the prostate due to an increase in stroma. In the prostates of this age period the tubules were again widely separated by stroma (Pl. 1, fig. 3).

Fully differentiated epithelium

Focal collections of tubules composed of tall columnar cells with clear reticular cytoplasm and basal nuclei, without perinuclear vacuolation, were seen in many prostates (Pl. 3, fig. 10). These tubules usually, but not always, possessed a basal layer of epithelial cells and were generally of the same size as the other tubules in the gland, though some were larger. Papillae were present in these tubules in four glands: in two of these—from a 38-week foetus and from a child aged 11 weeks—they were quite large.

This adult type of epithelium occurred most commonly in the lateral and posterior lobes, and only rarely in the anterior and middle lobes. It was not present in every prostate (Table 1), but its incidence and extent appeared to increase with maturity until term. After birth some regression occurred but this was not complete, and typical adult epithelium was seen at various ages during childhood.

Squamous metaplasia

The most striking histological feature of the prostates of the foetus and newborn was squamous metaplasia. The epithelium affected was composed of a deep layer of stratified cells, the superficial ones being swollen with large pale-staining nuclei and clear cytoplasm which was sometimes vacuolated. The deeper cell layers were compressed and composed of flattened cells with thin pyknotic nuclei. There was no evidence of keratin formation.

The metaplasia was only seen in its true form in groups 1–4, and of a total of forty-seven glands in these groups forty-five showed the change. The utricle, the urethra and the prostatic tubules near the urethra were the sites affected, but these varied and none was affected constantly. The utricle was affected in thirty glands and usually showed the change to a marked degree, becoming greatly distended, and the small tubules arising from it were also affected.

The urethra was affected in twenty-two prostates. The change was confined to the posterior wall and was usually very marked over the urethral crest, occasionally, however, only a few small scattered areas of metaplasia were present. It was most constant above the openings of the ejaculatory ducts, but occurred below these in five prostates. In addition to the metaplasia, and in some prostates without it, some of the urethral cells, including those in the anterior wall, were large with pale-staining nuclei and vacuolation of the cytoplasm which was either perinuclear or subnuclear. This was similar to the change in the epithelium of the tubules.

Squamous metaplasia occurred in the lateral lobes in thirty-eight prostates. The change was present symmetrically in the tubules close to their origin from the lateral furrows of the urethra and occasionally slight metaplasia also occurred in the tubules at the periphery of the gland. In the middle lobe metaplasia occurred in thirty-eight glands and was usually widespread, the tubules lying close to the ejaculatory ducts being commonly affected, even to the periphery of the lobe. In the anterior lobe metaplasia occurred in twenty-one prostates, it was always slight and confined to the origin of the tubules near the urethra.

In the posterior lobe metaplasia occurred in sixteen prostates. It was usually slight and occurred in two main situations, the first being in the tubules arising medially from the lower part of the urethral crest below the openings of the ejaculatory ducts, and the second in the very tip of the upper part of the lobe near the beginning of the ejaculatory ducts (Pl. 4, fig. 11).

In the prostatic tubules the metaplasia usually affected the whole circumference of the wall, distending and partially or completely occluding them. In some tubules, however, the change was slight, producing a small circular area of metaplasia in the lining epithelium; it appeared to begin beneath the luminal layer of cells.

Squamous metaplasia was present in the utricle and tubules of the anterior and lateral lobes of the prostate of the 24-week foetus and, in general, increased both in degree and extent with age up to term. It remained until the seventh day of life and then decreased. The superficial cells desquamated and the basal cells were replaced by stratified columnar or transitional epithelium. This process was complete by the fourth week of life except in the utricle which, following desquamation of the superficial cells, remained distended until the fourth month and lined by low stratified epithelium until just before puberty when this was replaced by stratified columnar epithelium. There was, however, some variation in the incidence and degree of the metaplasia (Table 1). In two prostates it was absent and in a few others it was very slight, but in some glands it was very marked, producing great distension of the tubules and completely blocking them (Pl. 4, fig. 12). In one prostate a group of these distended tubules formed a small nodule.

Following desquamation of the squamous epithelium the distended tubules usually returned to their normal size but in one prostate, removed from a boy aged $5\frac{1}{2}$ years,

cystic tubules lined by flattened epithelium and containing eosinophil secretion were present in sites where squamous metaplasia usually occurred, and it seemed possible that these cysts were in some way connected with the preceding metaplasia.

Cyst formation

In addition to the dilatation of the tubules produced by the metaplasia, other parts of the tubules were also dilated (Pl. 4, fig. 13). These dilated tubules pressed on one another so that their adjacent walls became thin and ruptured, and the tubules united, producing cysts lined by an epithelium which was rather lower than that of the rest of the tubules.

The dilatation may possibly have been the result of a damming back of secretion through obstruction produced by the metaplasia lower down the tubules, but there was little evidence of retained secretion in the cysts in the paraffin sections and the tubules remained dilated until the fourth month, which was after the metaplasia had disappeared.

The cysts had no regular distribution within the prostate. All lobes were affected, although not usually in the same gland. The cysts were not present in every gland (Table 1), and it was not possible to establish a direct connexion between the cysts and any of the other changes.

The ejaculatory ducts

During the later months of foetal life the ejaculatory ducts showed some degree of hyperplasia. The epithelium, which was composed of large eosinophil cells, was thrown up into papillae especially near the urethra, and the ducts were markedly dilated. Eosinophil secretion was present in the lumen. The incidence of this change was variable (Table 1), but its degree appeared to be roughly related to the squamous metaplasia and the epithelial changes. The ejaculatory ducts remained dilated until the eleventh post-natal month.

Corpora amylacea

Small corpora amylacea were present in many of the prostates. In the youngest glands they were in the termination of the tubules near the urethra and appeared to be due to accretion and inspissation of the eosinophil secretion. By 16 months and in older glands they were more widely distributed. A small concretion was present in the utricle of the 24-week foetus, and in the prostate of a child of $2\frac{1}{2}$ years the corpora amylacea appeared to contain calcium salts.

Elastic tissue

The elastic tissue in the prostates studied was relatively scanty. It consisted of fine fibrils intimately mixed with the supporting stroma. There was, however, a considerable condensation of the elastic tissue around the ejaculatory ducts, the utricle and the urethra, including the origins of the tubules; it was separated from the latter by connective tissue and bore no direct relationship to the epithelium.

Elastic tissue was present in the foetal prostates in the sites described and, except perhaps for a slight increase, no alteration occurred up to the age of 13 years.

Breast feeding

Breast feeding apparently had no influence upon any of the changes described.

The specific hyperplasia previously described in adult prostates (Andrews, 1949) was not seen in the prostates of the foetus and child, neither were any changes seen which could be held to portend the development of carcinoma in later life.

Gross anatomy

The practice of considering the prostate as divided into five lobes (Lowsley, 1930) provides a convenient terminology for the rough siting of lesions, but, in the present series, such a division appeared to be artificial. The lobes were not sharply demarcated and the posterior lobe did not possess a capsule. Thus the site of the various lesions could only be roughly determined by studying their relationship to the urethra and ejaculatory ducts.

The main prostatic tubules arose symmetrically from the lateral furrows of the urethra and ran a curved course posteriorly, laterally and finally anteriorly, into the lateral lobes. These tubules gave off branches along their course. Another important but narrower set of tubules arose symmetrically from the posterior wall of the urethra slightly medial to the lateral furrows. These ran a course parallel to the first set of tubules and also gave off branches from which it is probable that some of the acini of the posterior part of the gland are formed. In addition to these two main sets of tubules, shorter tubules arose from the anterior, lateral and posterior walls of the urethra. Those arising from the posterior wall immediately above and below the openings of the ejaculatory ducts followed the line of these ducts upwards and backwards.

DISCUSSION

It is concluded that during the last months of pregnancy the foetal prostate shows proliferation and alteration of the tubule epithelium, differentiation of adult epithelium, squamous metaplasia, cystic dilatation of the tubules and hyperplasia and dilatation of the ejaculatory ducts. These changes increase both in degree and extent until term, persist for 1–4 weeks after birth and then undergo a gradual regression, which may be complete or partial, over a period of 1–4 months, although the ejaculatory ducts usually remain dilated for longer. These changes are roughly co-ordinated.

There may be variation between prostates of the same age period, in some the changes may be slight whilst in others they may be very marked. There may also be variation in the individual changes within a single prostate, thus one or more of the changes may be present and the rest absent. The proliferation and alteration of the epithelium and squamous metaplasia are the most constant changes, and the adult epithelium, cystic dilatation of the tubules and hyperplasia of the ejaculatory ducts are the most variable. No confirmation was obtained of the statement by Moore (1936) that the adult epithelium failed to appear in the prostates of premature infants.

These changes in the immature prostate occur at a time when changes also occur in the breast (Dawson, 1934), uterus (Scammon, 1923) and vagina (Fraenkel &

Papanicolau, 1938) of the foetus, and it is suggested that all these changes are due to the action of oestrogens. Squamous metaplasia has been produced experimentally in the prostates of mice (Lisco & Biskind, 1941) and monkeys (Parkes & Zuckerman, 1935) by the injection of oestrogens, and its normal regression in the prostate of the newborn child has been delayed by the injection of oestrogens (Sharpey Schafer & Zuckerman, 1941). Moore & McLellan (1938) have also produced squamous metaplasia in the adult human prostate by the action of oestrogens. It has been shown by Smith & Smith (1937) that the oestrogen level in the maternal serum rises during the later months of pregnancy, and Soule (1938) has found equal concentrations of oestrogens in maternal and foetal blood. It is almost certain, therefore, that the squamous metaplasia is due to maternal oestrogens passing in increasing amounts through the placenta during the later months of pregnancy. The increase in degree and extent of the metaplasia with maturity in the prostates of premature infants agrees with this.

There was a close co-ordination between the appearance of the squamous metaplasia and the presence of perinuclear vacuolation in the cells of the tubules and, in addition, there was a close similarity in histological appearance between the metaplastic cells and the vacuolated cells. It seems most probable that oestrogens also produce this vacuolation, particularly as Kahle, Schenken & Burns (1943) have described vacuolation of the cytoplasm in the cells of carcinomata of the prostate treated with oestrogens. However, despite a rough co-ordination between the squamous metaplasia and the other changes it is unlikely that oestrogens produce all of them. In experimental work on the action of oestrogens on the prostates of animals the only reference to changes other than squamous metaplasia is made by Parkes & Zuckerman (1935) who state that, in the monkey, oestrogens cause narrowing of the prostatic tubules and a reduction in their number. This is the exact opposite of the behaviour of the tubules in the human foetal prostate. Although it cannot be assumed that oestrogens will have the same effect on human as on animal tissue, it is unlikely that, acting on 'male' tissue, they would produce proliferation of the prostatic epithelium, fully differentiated adult epithelium and hyperplasia of the ejaculatory ducts, especially as it is known that the growth of the prostate at puberty is due to the action of androgenic hormones. It seems probable, therefore, that additional factors are acting on the immature prostate. These may be maternal androgenic hormones acting directly on the foetal prostate or maternal pituitary hormones which primarily stimulate the testis of the foetus to produce androgenic hormones which then act on the prostate. It has been shown by Price (1947) that activity of the prostate in suckling rats depends on two conditions, the first being intact ovaries in the mother, which are necessary for the growth of the prostate, and the second, intact testes in the suckling, which are necessary for differentiation of the prostatic epithelium.

The variations that occurred between prostates of the same age group may be due to differences in concentration of the stimuli, but the variation in the distribution of some of the changes in prostates that were otherwise the same indicates that there is a definite difference in the response of the individual cells. This is shown most markedly in the focal distribution of the fully differentiated epithelium and the cystic dilatation of the tubules. Moore (1936) compared this focal change with the

focal proliferation occurring in benign hypertrophy. There is an undoubted similarity between the changes in the immature prostates and those of benign hypertrophy, but whereas in the latter the proliferation is focal with the formation of multiple nodules, in the immature prostates, although the differentiation is focal, the proliferation is usually uniform and diffuse. Only two small nodules were seen; one of these being the direct result of squamous metaplasia. In benign hypertrophy the changes seldom occur in the posterior lobe, but in the immature prostates they occurred in all lobes. There was no evidence to suggest that the posterior lobe is a separate structure either anatomically or physiologically (MacLeod, 1942; Moore, 1943).

The variation in the histological appearance of the prostatic tubules during the later months of foetal life was an interesting feature of this study. They were commonly formed of two layers of cells, a luminal layer and a horizontal basal layer, but many possessed a luminal layer only. The luminal layers were composed of either low columnar or flattened epithelium. This difference in height was quite clear-cut and there appeared to be no gradation between the two types. Both had the same cytoplasmic appearances and it was therefore considered that they did not represent different stages of a cycle. There was also a variation in the size of the tubules, some of them being cystic. These cysts were more commonly lined by the flattened type of epithelium and were formed by the joining of dilated tubules without preceding proliferation and desquamation of epithelium. It remains to be shown whether this variation in pattern is a normal differentiation produced by androgenic hormones or an abnormal feature caused by oestrogens.

SUMMARY

1. The result of a histological study of sixty-six prostates removed at autopsy from infants and children between the twenty-fourth week of foetal life and 13 years of age is reported.

2. Squamous metaplasia, proliferation and alteration of the epithelium of the tubules, fully differentiated adult epithelium, cystic dilatation of the tubules and hyperplasia and dilatation of the ejaculatory ducts occurred in the majority of the prostates and increased in degree during the last months of foetal life up to term. The changes persisted for 1–4 weeks and gradually regressed over 1–4 months.

3. The possible aetiology of these changes, and their relationship to benign hypertrophy is discussed.

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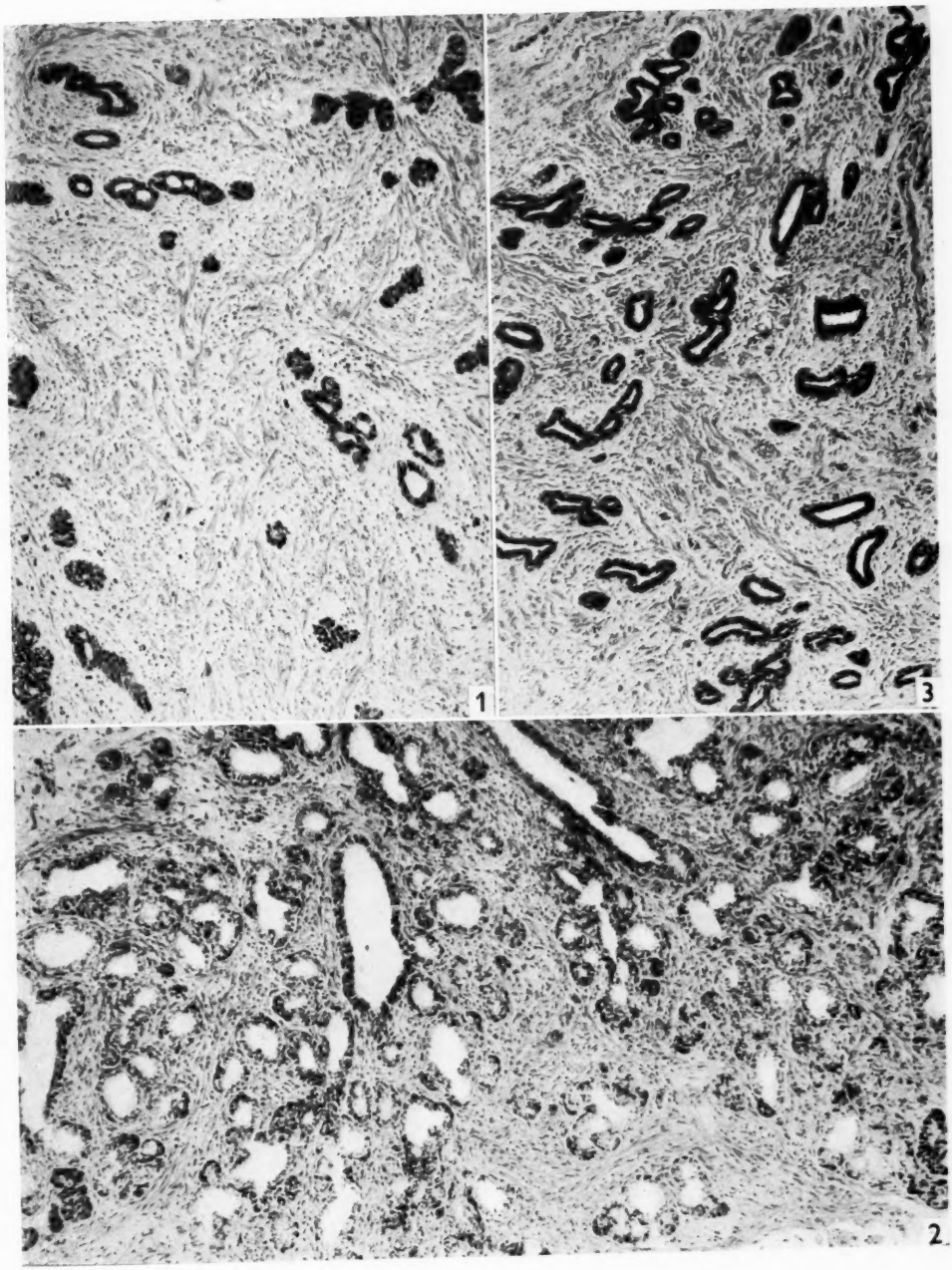
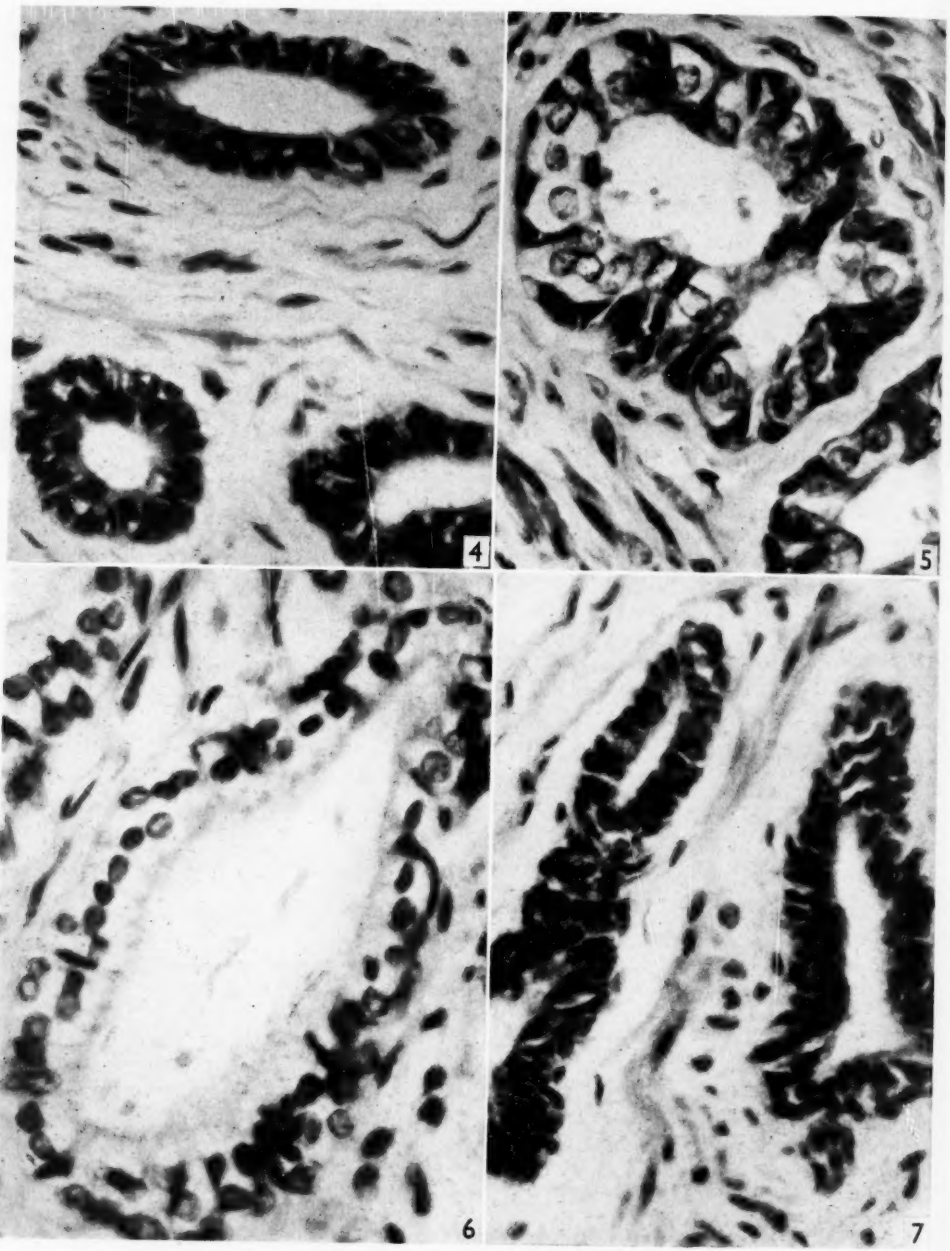


ANDREWS—HISTOLOGY OF HUMAN FOETAL AND PREPUBERTAL PROSTATES



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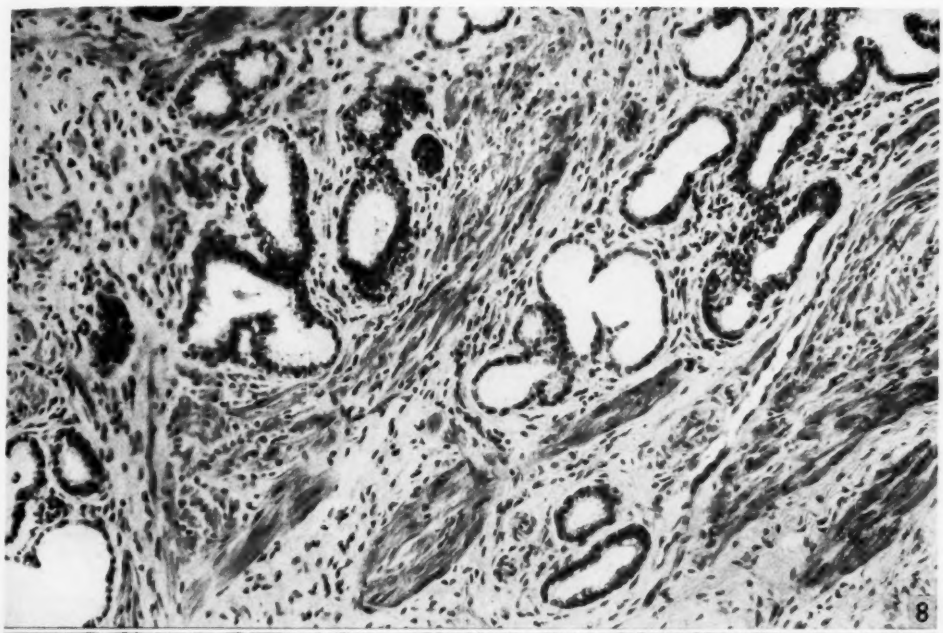
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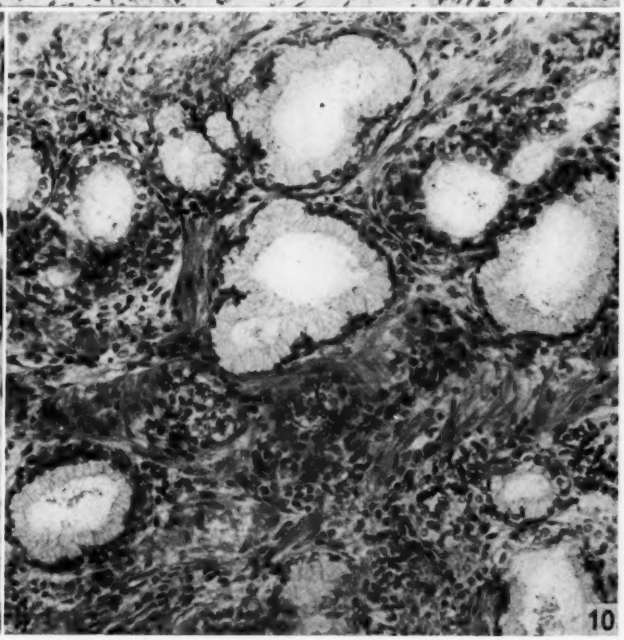
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ANDREWS—HISTOLOGY OF HUMAN FOETAL AND PREPUBERTAL PROSTATES

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Prostate from a 24-week foetus showing few tubules, the majority of which are solid and composed of small darkly staining cells, separated widely by stroma.

Fig. 2. Prostate from a full-term infant. The tubules have increased in number and lie closer together, they are composed of large cells with pale-staining nuclei and clear cytoplasm.

Fig. 3. Prostate from a child aged 2½ years. The tubules are separated by stroma and composed of darkly staining cells. All haematoxylin and eosin. ×100.

PLATE 2

Fig. 4. Prostate from a 24-week foetus. The tubules are composed of cells with pyknotic nuclei.

Fig. 5. Prostate from a 33-week foetus. The majority of tubule cells are larger than those of the 24-week foetus and perinuclear vacuolation is present. The nuclei are mainly large and pale-staining but pyknotic nuclei are still present.

Fig. 6. Prostate from a full-term infant. The tubule cells are taller than those in Pl. 2, figs. 4 and 5. They possess large pale nuclei and show perinuclear vacuolation.

Fig. 7. Prostate from a child aged 2½ years. The tubule cells possess pyknotic nuclei and there has been a return to the cellular appearance of the 24-week foetus. All haematoxylin and eosin. ×800.

PLATE 3

Fig. 8. The prostate of a child aged 3 months showing variation in the height of the epithelium. Haematoxylin and eosin. ×200.

Fig. 9. A tubule from the prostate of a 38-week foetus lined by low epithelium with large nuclei and perinuclear vacuolation. The basal layer of epithelial cells is absent. Haematoxylin and eosin. ×800.

Fig. 10. Fully differentiated epithelium in the prostate of a 38-week foetus. Haematoxylin and eosin. ×200.

PLATE 4

Fig. 11. Sagittal section showing squamous metaplasia in the apex of the posterior lobe behind the ejaculatory ducts (E.D.). Haematoxylin and eosin. ×75.

Fig. 12. Marked squamous metaplasia in the prostate of a 38-week foetus. Haematoxylin and eosin. ×50.

Fig. 13. Cystic tubules in the prostate of an infant aged 3 weeks. Haematoxylin and eosin. ×75.

THE MAXIMUM TORQUES DEVELOPED IN PRONATION AND SUPINATION OF THE RIGHT HAND

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As part of an investigation on the strength and amplitude of joint movement in human subjects a study has been made of the effect of different hand, elbow and shoulder positions on the maximum *static* force (more accurately isometric torque) that can be exerted in attempted pronation and supination of the hand. In order to record these torques, a dynamometer has been developed, making use of electrical strain gauges.

Although numerous investigations have previously been made on the muscular strength of human subjects by means of dynamometers in connexion with various problems, there is little information on the effect of joint-position on the force developed. Such information as is available is derived from the work of Braune & Fischer (1890), Franke (1920), Hansen & Lindhard (1923), Hvorslev (1928), Garry (1930), Müller (1935), Haxton (1945), Dern, Levene & Blair (1947), Hugh-Jones (1947), Clarke, Elkins, Martin & Wakim (1950), Wakim, Gersten, Elkins & Martin (1950) and Wilkie (1950). The majority of these studies are confined to flexion of the elbow and extension of the knee, and the only references in the literature on the force of the movements in pronation and supination of the hand are contained in papers by Bethe & Franke (1919) and Reijs (1921).

METHODS

Measurements. The maximum torque that can be developed isometrically in attempting to turn the right hand in an anti-clockwise direction towards its fully pronated position (pronation torque), or in a clockwise direction towards its fully supinated position (supination torque), was determined with the hand in various positions between full pronation and full supination. These measurements were carried out with the shoulder and elbow in six different positions (see Table 1).

Table 1. *The shoulder-elbow position investigated and the zero position of the hand*

	Shoulder position	Elbow position	Forearm position	Hand position
A	Adducted to the side of the body	Fully extended	Vertical	Thumb anterior, palm facing medially
B	Adducted to the side of the body	Flexed at 90°	Horizontal	Thumb superior, palm facing medially
C	Flexed in the sagittal plane at 90°	Fully extended	Horizontal	Thumb superior, palm facing medially
D	Flexed in the sagittal plane at 90°	Flexed at 90°	Vertical	Thumb posterior, palm facing medially
E	Abducted in the coronal plane at 90°	Fully extended	Horizontal	Thumb anterior, palm facing inferiorly
F	Abducted in the coronal plane at 90°	Flexed at 90°	Horizontal	Thumb medial, palm facing inferiorly

Since there is no generally accepted method of recording the degrees of rotation of the hand resulting from movements of the radio-ulnar joints, and also, if the elbow is extended, from rotation of the humerus, for the purposes of these experiments each position of the hand shown in Table 1 was taken as zero. This position was not necessarily the mid-point between the fully pronated position and the fully supinated position of the hand. Angular displacements of the right hand from this position in an anti-clockwise (pronation) direction were recorded as minus and in a clockwise (supination) direction as plus. Data were obtained with the hand in the following positions: $-75°$, $-45°$, $-15°$, $+15°$, $+45°$, $+75°$ and $+105°$. The subjects were unable to reach the extreme angles in certain positions of the shoulder and elbow but, in all positions, the range tested was between $-45°$ and $+75°$.

Apparatus. A strain gauge dynamometer was used to measure the maximum torques (Pl. 1, fig. 1). It consists of a wooden handle connected by a metal stirrup to a tubular metal spindle, which passes perpendicularly through a ball-race bearing in the centre of a rigid metal plate. A tempered steel bar traverses the spindle immediately in front of the plate. Both ends of the bar can be firmly fixed to the plate by metal pegs in angular positions at 30° intervals. The hand-grip spindle is provided with a pointer which indicates, on a scale attached to the metal plate, the angular position of the handle. The whole apparatus is mounted so that it can be tilted in all directions and adjusted in height.

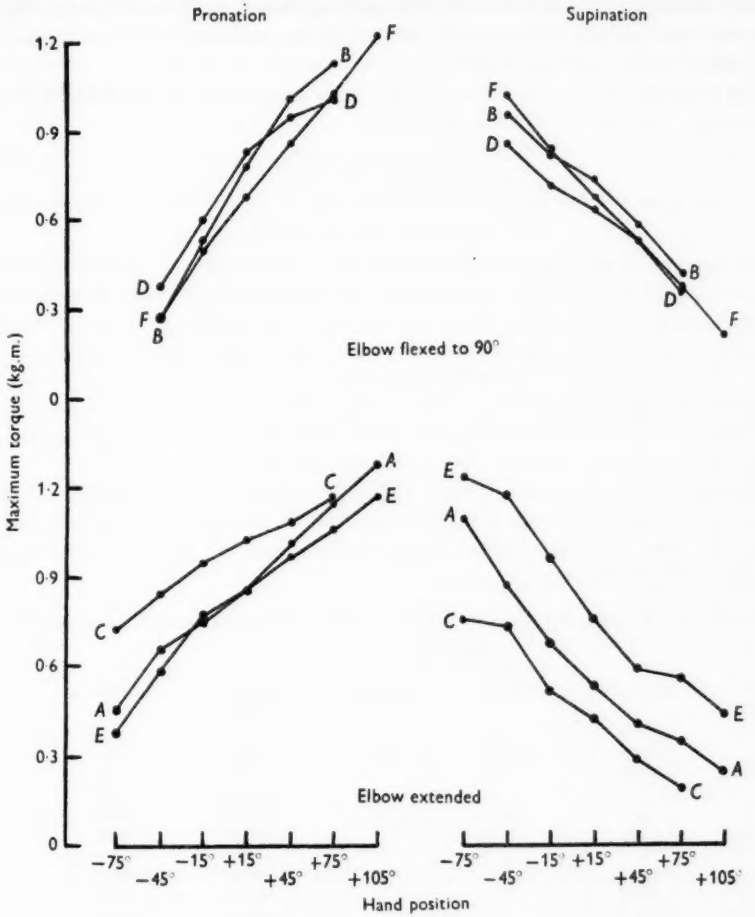
The strength of the tempered steel is such that the application of a torque of as much as 1 kg./m. causes an angular rotation of the handle of only 1°. On attempting to twist the handle, in either a clockwise (supination) or an anti-clockwise (pronation) direction, the tempered steel bar is, nevertheless, slightly deformed. The degree of the deformation is measured by two paper-mounted 1000 Ω resistance strain gauges fixed to the upper surface of the tempered steel bar on opposite sides of the hand-grip spindle. Thus, the application of a torque causes one strain gauge to be extended and simultaneously the other to be contracted. The gauges form two arms of a Wheatstone Bridge, the output of which is taken to a galvanometer (sensitivity: 1 μA./25 mm.). Facilities are provided for checking and adjusting the voltage applied to the bridge and for calibrating the dynamometer by the application of known torques. Calibration of the apparatus was carried out immediately before and after every experiment.

Experimental subjects. The subjects were three naval ratings, aged 18, 23 and 25, of apparently normal physical development. Each was right-handed. None of them had had any previous experience of the apparatus.

Design of the experiment. The final design was based on pilot experiments using six subjects who did not take part in the present series.

Each subject was seated in a chair in a comfortable erect posture and the height and tilt of the dynamometer were adjusted so that the handle could be grasped by the right hand with the shoulder and elbow in the desired position. During pronation and supination the hand rotates around an axis passing through its centre (Duchenne, 1867). In all positions, therefore, the right forearm was arranged to be in line with the spindle of the dynamometer, so that the axis of movement of pronation and supination corresponded with the axis of the movement of the dynamometer spindle. In each of the six shoulder-elbow positions the *range* of the movement

between full supination and full pronation (the range of pronation and supination) was first measured. The handle was then *fixed* in the initial hand position and the subject instructed to exert as great a force as rapidly as possible, either in a clockwise (supination) or anti-clockwise (pronation) direction without moving the arm or the



Text-fig. 1. Maximum torques exerted in different hand positions. Average of thirty observations on the three subjects. *A* and *B*, shoulder adducted; *C* and *D*, shoulder flexed to 90°; *E* and *F*, shoulder abducted to 90°.

trunk. The maximum torque of pronation and supination was then recorded in as many of the hand positions between $-75°$ and $+105°$ as possible. The order in which the hand positions and the direction of the attempted movements were taken was randomized. This series was then repeated without an interval. Two different shoulder-elbow positions, selected at random, were studied daily. Thirty seconds elapsed between each exertion in each shoulder-elbow position, and 1 hr. between runs in

different shoulder-elbow positions. The subjects could neither see the galvanometer scale nor were they told of the results of their efforts throughout the whole experiment. The positioning of the subject and recording of the galvanometer readings were always performed by the same observers.

The whole experiment lasted 15 days, in three 5-day periods with 2-day intervals between each period. During the course of the experiment, the temperature of the laboratory varied from 13 to 20° C.

The readings between −45° and +75° hand position in all shoulder-elbow positions were subjected to statistical analysis.

OBSERVATIONS

The effect of hand position on the maximum torques developed in attempted pronation and supination

In the total range of hand positions investigated, there is a linear relationship between the position of the hand and the isometric torque developed. The correlation coefficients were all significant to below the 0·01 level of confidence, and the regression lines made a very close approximation to the lines drawn from the raw data.

It will be observed from Text-fig. 1 that the isometric torque of pronation increases and that of supination decreases as the position of the hand alters in the direction of supination (i.e. with clockwise rotation of the right hand). The slope of the pronation curve is thus positive and that of the supination curve negative.

The slope of the pronation curve is slightly steeper than that of the supination curve, except when the elbow is extended and the shoulder is flexed or abducted, when the opposite is true. Table 2 gives the average difference of the torques in successive hand positions.

Table 2. *Average difference in torque exerted between successive hand positions*

Subject	Position: Elbow	Position: Shoulder	Pronation (kg.m.)	Supination (kg.m.)
I	Ext.	Add.	0·090	0·165
II			0·150	0·069
III			0·135	0·102
I	Flex.	Add.	0·285	0·207
II			0·156	0·105
III			0·216	0·084
I	Ext.	Flex.	0·078	0·162
II			0·098	0·099
III			0·054	0·075
I	Flex.	Flex.	0·256	0·171
II			0·045	0·096
III			0·171	0·090
I	Ext.	Abd.	0·144	0·204
II			0·090	0·126
III			0·114	0·180
I	Flex.	Abd.	0·255	0·240
II			0·141	0·132
III			0·186	0·108

For pronation, the slope of the curve is steeper when the elbow is flexed than when it is extended. The slope of the supination curve appears to be independent of the position of the elbow.

In general, the pronation torque is greater than the supination torque at the supination end of the range tested; the reverse holding at the pronation end. (An exception to this was found in one subject, in whom in one shoulder-elbow position the pronation torque exceeded the supination torque in all hand positions.) At some intermediate hand position, therefore, the pronation and supination torques will be equal. These positions have been summarized in Table 3. It will be noted that there is considerable variation in the positions of equal torque with the different shoulder-elbow positions.

Table 3. *The hand positions in each shoulder-elbow position at which the torque of pronation equals that of supination*

Position		Subjects		
Elbow	Shoulder	I (°)	II (°)	III (°)
Ext.	Add. (*A*)	−27	−8	−33
	Flex. (*C*)	−67	−50	(−104)*
	Abd. (*E*)	+16	+12	−4
Flex.	Add. (*B*)	+14	+13	+8
	Flex. (*D*)	+7	−27	+6
	Abd. (*F*)	+17	+22	+18

* This figure has been obtained by extrapolation as it was beyond the range of hand positions tested.

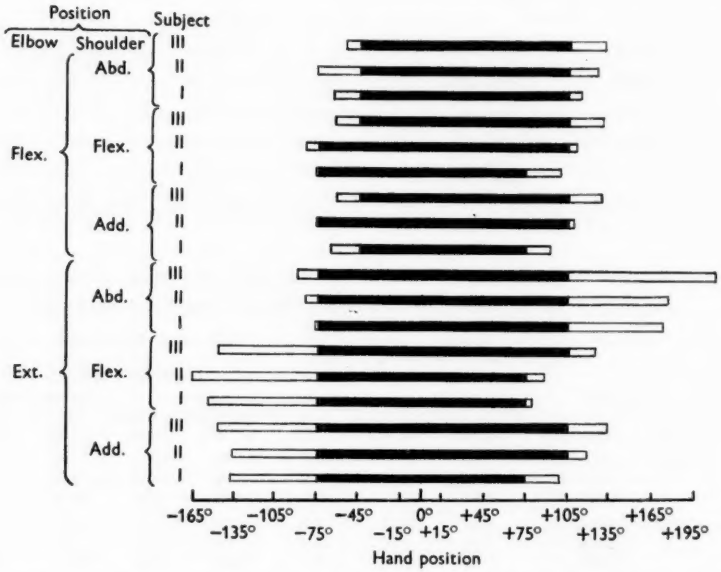
Table 4. *Average torque of pronation and supination for the range of hand positions −45° to +75° and at the mid-point of the full range of movement*

			Range −45° to +75°			Mid-point of full range			
	Position		Average torque		Difference (supination ≡100)	Average torque		Difference (supination ≡100)	Mid-point of full range
Subject	Elbow	Shoulder	Pronation (kg.m.)	Supination (kg.m.)		Pronation (kg.m.)	Supination (kg.m.)		
I	Ext.	Add. (*A*)	1·03	0·67	53·7	0·91	0·85	7·1	−18·6
		Flex. (*C*)	1·17	0·46	154·3	0·98	0·72	36·1	−37·2
		Abd. (*E*)	0·94	0·94	—	1·00	0·72	38·9	+48·8
	Flex.	Add. (*B*)	0·78	0·77	1·3	0·76	0·77	−1·3	+12·5
		Flex. (*D*)	0·81	0·70	15·7	0·78	0·72	8·3	+11·4
		Abd. (*F*)	0·75	0·76	−1·3	0·82	0·69	18·8	+24·4
II	Ext.	Add. (*A*)	0·70	0·54	29·6	0·60	0·59	1·7	−6·7
		Flex. (*C*)	0·85	0·42	102·4	0·67	0·60	11·7	−39·4
		Abd. (*E*)	0·72	0·74	−2·7	0·81	0·61	32·8	+46·1
	Flex.	Add. (*B*)	0·70	0·67	4·5	0·70	0·67	4·5	+16·8
		Flex. (*D*)	0·73	0·53	37·7	0·73	0·53	37·7	+13·9
		Abd. (*F*)	0·57	0·62	−8·1	0·61	0·58	5·2	+24·5
III	Ext.	Add. (*A*)	0·94	0·48	95·8	0·85	0·56	51·8	−8·5
		Flex. (*C*)	1·00	0·39	156·4	0·95	0·45	111·1	−12·0
		Abd. (*E*)	0·89	0·70	27·1	1·04	0·44	136·4	+57·6
	Flex.	Add. (*B*)	0·75	0·68	10·3	0·88	0·62	41·9	+33·4
		Flex. (*D*)	0·71	0·64	10·9	0·83	0·58	43·1	+34·5
		Abd. (*F*)	0·67	0·71	−5·6	0·81	0·63	28·6	+37·3
Total for all positions			0·82	0·63	30·2	0·82	0·63	30·2	—
Total for all positions with elbow extended			0·92	0·59	55·9	0·87	0·62	40·3	—
Total for all positions with elbow flexed			0·72	0·68	5·9	0·77	0·64	20·3	—

The effect of shoulder-elbow position on the 'average maximum torque' in attempted pronation and supination

The 'average maximum torque' is the mean of torques in all hand positions within the common range for all runs in a particular shoulder-elbow position. The effect of shoulder-elbow position on the 'average maximum torque' in pronation and supination is summarized in Table 4.

For the common range of hand position ($-45°$ to $+75°$), it will be noted that the average maximum torque varies with the different shoulder-elbow positions in both pronation and supination. In the majority of instances, the pronation torque exceeds the supination torque, the difference being largest when the elbow is extended



Text-fig. 2. Diagram illustrating the part of the full range tested in each shoulder-elbow position. □, full range; ■, range tested.

and the shoulder flexed. The supination torque is greater than the pronation torque in only four cases, in three of which the elbow is flexed and the shoulder abducted. These differences, however, are insignificant.

Table 4 also indicates that the mid-point of the common range tested (i.e. $+15°$) does not correspond with the mid-point of the full range of pronation and supination. Since the torque of pronation is becoming greater as the hand position approaches full supination, the apparent general superiority of the pronation torques may be due to the fact that, of the hand positions tested, more lie on the supination side of the mid-point of the full range of movement than on the pronation side.

If it can be assumed that the linear relationship found between the isometric torques developed and the hand position tested holds for the full range, then a more

representative estimate of the relative strengths of pronation and supination may be obtained by calculating the torques exerted at the mid-points of the range for each shoulder-elbow position. With the elbow *flexed*, practically the whole range has been included (Text-fig. 2) showing a linear relationship, so that there is little doubt that a linear relationship would also hold had the extreme positions been included. However, with the elbow *extended*, the range tested showed a bias either towards the supination or the pronation end of the full range. It may be assumed that had the whole range been tested a linear relationship would still have been found, because if deviation from the relationship does not occur at one end of the full range in one particular shoulder position, it is hardly likely that it would have been found if the same part of the full range had been tested in another shoulder position.

At the mid-point of the range of pronation and supination the total of the maximum torques is greater for pronation than supination (Table 4). In twelve of the eighteen shoulder-elbow positions, pronation is more than 10 % greater than supination. In only one position in one subject does supination exceed pronation and then only by 1·3 %, a difference which is not significant. The greatest differences between pronation and supination are found when the elbow is extended and, particularly, when the shoulder is also abducted. The least difference occurs when the shoulder is adducted and the elbow flexed. Between the different shoulder-elbow positions greater variations in the maximum torques are found for both pronation and supination when the elbow is extended than when it is flexed.

Variations in the maximum torques developed in the same shoulder-elbow position

In each of the shoulder-elbow positions there is no consistent improvement or falling off in performance either in pronation or in supination in successive runs (Text-fig. 3). A significant improvement is found in only two of the thirty-six runs and a falling off in two; the significance of the correlation coefficients in these instances being below the 0·02 level of confidence. In the other thirty-two positions there is no correlation between total maximum torque and the order of the runs.

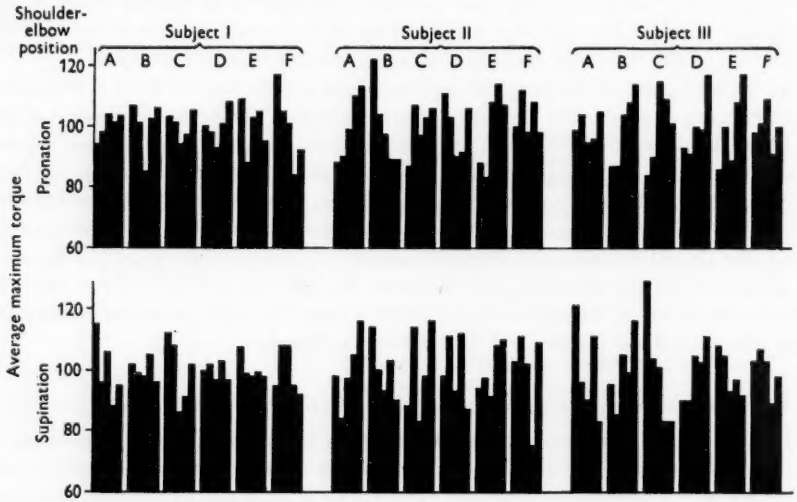
The variations of the total maximum torques in pronation and in supination from the average for each position are greatest in those shoulder-elbow positions in which the total maximum torque was smallest (Table 5). Similarly, in any one shoulder-elbow position, the variation was greatest in the weakest hand positions (Text-fig. 4).

Table 5. *Average torques and the coefficient of variations for the five runs in each shoulder elbow-position for pronation and supination*

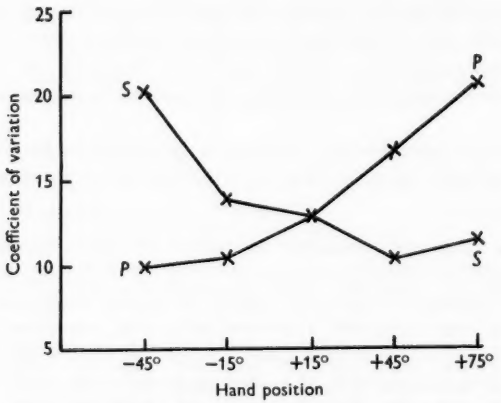
	Subject I				Subject II				Subject III				All subjects	
	Pronation		Supination		Pronation		Supination		Pronation		Supination			
Shoulder-elbow position	Av. (kg.m.)	C. of V.	Av. (kg.m.)	C. of V.	Av. (kg.m.)	C. of V.	Av. (kg.m.)	C. of V.	Av. (kg.m.)	C. of V.	Av. (kg.m.)	C. of V.	*P* Av.	*S* Av.
A	1·03	4·2	0·67	10·1	0·70	10·9	0·54	12·1	0·94	4·5	0·48	15·1	0·98	0·56
B	0·78	8·8	0·77	3·7	0·70	13·4	0·67	8·9	0·75	12·4	0·68	11·6	0·74	0·70
C	1·17	4·5	0·46	11·1	0·85	7·8	0·42	14·7	1·00	12·7	0·39	19·1	1·00	0·42
D	0·81	5·4	0·70	2·3	0·73	9·3	0·53	11·6	0·71	9·9	0·64	9·2	0·75	0·62
E	0·94	8·8	0·94	3·0	0·72	13·6	0·74	8·3	0·89	12·8	0·70	7·1	0·85	0·79
F	0·75	13·1	0·76	7·5	0·57	10·4	0·62	14·1	0·67	6·5	0·71	6·6	0·66	0·69
Av.	0·91	—	0·72	—	0·71	—	0·58	—	0·83	—	0·60	—	0·82	0·63

There was no relationship between the torque exerted in pronation and supination in the same run in the same shoulder-elbow position. For instance, if the total pronation torque in any one shoulder-elbow position in one run was above the average for the five runs in that position, the total supination torque did not necessarily vary in the same direction (Text-fig. 3).

However, there is no significant difference between the total maximum torques in the same shoulder-elbow position in the first series of recordings and the second taken immediately after the first.



Text-fig. 3. The total maximum torque for each run in each shoulder-elbow position expressed as a percentage of the average for each position. Each histogram represents the five consecutive runs in each shoulder-elbow position.



Text-fig. 4. The average coefficient of variation for each hand position in all shoulder-elbow positions. *P–P*, pronation, *S–S* supination.

DISCUSSION

The effect of hand position on the maximum torque. Several factors may be responsible for the changes observed in the torque of pronation and supination in different hand positions. These are the degree of shortening of the prime movers, the mechanical advantage of the muscles, the extent of their contraction, the number of muscles involved, and the activity of the antagonists.

It is well-known that the contractile force of a muscle is reduced as it shortens (Evans & Hill, 1914; Hartree & Hill, 1921; Stephens & Metcalf, 1934; Arkin, 1941). Therefore, as the hand is pronated, the torque exerted by the pronator muscles will be reduced and that exerted by the supinator muscles increased, and the reverse will occur as the hand is supinated.

The effect is usually overshadowed by the changes in the mechanical advantage of the muscles. For instance, in flexion of the elbow, as the flexor muscles shorten the lever arm of these muscles increases until the elbow is bent to a right angle. During this phase, the force exerted increases in spite of the diminished length of the muscles. Further flexion of the elbow, on the other hand, causes a reduction in the lever arm and the force of flexion is reduced. This is, to some extent, accentuated by the continued shortening of the muscles. In this example, the greatest strength occurs in the middle of the range (Bethe & Franke, 1919; Franke, 1920; Reijs, 1921; Wakim *et al.* 1950).

The changes in torque during pronation and supination, however, follow a different pattern, for, as has been shown, there is a continuous rise or fall in torque throughout the range of movement. This may be partly explained by a study of the muscles effecting the movements. When the elbow is flexed, pronation and supination are brought about by muscles acting on the radio-ulnar joints. When the elbow is extended, the rotator muscles of the scapulo-humeral joint are also called into play. These muscles are inserted so that at their maximum length they wrap themselves around the bone on which they are acting. Thus for a variable degree of the movement, depending upon the extent of this 'wrapping', the leverage of the muscles remains constant, for the perpendicular distance of the line of force from the axis of the movement does not alter. When the muscles have become unwrapped, their mechanical advantage falls rapidly. Thus one might expect a more rapid reduction in torque at the end of the range, when there is a reduced leverage, as well as shortening of the muscles.

The fact that, instead, a linear fall in torque was found may be accounted for by a variation in the activity of the muscles taking part. It has been assumed above that the muscles are all contracting maximally and that all are contracting in all phases of the movement. Dempster & Finerty (1947) have shown, as a result of electromyographic studies of the muscles acting on the wrist, that in this case at least, the activity of muscles is much greater when they are in the position of greatest mechanical advantage than other positions. Similarly, the results of a study of the shoulder muscles by Inman, Saunders & Abbott (1944) indicate that the electrical activity of these muscles varies with the position of the joint on which they are acting. It is also possible that all the agonist muscles are not contracting simultaneously. It has been suggested, for instance, that in supination at the radio-ulnar joints the supinator

muscle alone is responsible for the power of this movement when the hand is almost fully supinated (Steindler, 1935).

The effect of hand position on the isometric torque of pronation and supination may be further modified by the action of the antagonist muscles. However, examination by palpation of the pronator and supinator muscles during maximal contraction indicates that in attempted pronation the biceps muscle is relaxed, and in attempted supination the pronator teres muscle is relaxed. This is supported by the findings of Beevor (1891, 1904) on palpation, and of Hoefer (1941) and Wilkie (1950) on electromyographic examination that, in movement against an external resistance, there is relaxation of the antagonist. On the other hand, Dern *et al.* (1947) state that there is considerable antagonistic activity in isometric contraction (although they did find that in one subject the biceps was electrically inactive during isometric extension), and Golla & Hertwer (1924) found that, in supination and pronation against resistance, electrical activity can be recorded from both the biceps and pronator teres muscles, although the agonists show a greater amplitude of oscillations on the electromyogram.

From this discussion, it can only be concluded that further investigations are necessary to decide the relative importance of the factors influencing the forces that can be exerted by muscles in the intact body.

The effect of shoulder-elbow position on the total maximum torques. It is generally stated in text-books of Anatomy that supination is a much more powerful movement than pronation (Morris, 1902; Muller, 1929; Walmsley, 1934; Johnston, 1946; Mainland, 1947). The only reports of original work on the torque of pronation and supination that have been found are those of Bethe & Franke (1919) and Reijs (1921). Bethe & Franke do not state in which shoulder-elbow position they have determined the torques of pronation and supination but, presumably, it was with the elbow extended as their range of hand positions was over 200°. Their graphs indicate that the average maximum torque is greater for pronation than supination, although they do not comment on this themselves. Reijs studied the torque of pronation and supination with the elbow flexed in eight subjects. However, he gives no details of his experiments. The graphs, which are said to be drawn in the same proportion for pronation and supination, indicate that the average supination torque throughout the range is much greater than the pronation torque. The only position in which pronation approaches supination is when the hand is fully supinated, a position in which one would expect pronation to exceed supination. In the present series of experiments, in only one shoulder-elbow position does the average strength of supination exceed that of pronation and then only to an insignificant degree. There are certain positions of the hand in all shoulder-elbow positions where the strength of supination is greater than that of pronation, but taking the average of the whole range pronation is stronger than supination.

The finding that there is less variation in the maximum torques developed in different positions of the shoulder with the elbow flexed than with the elbow extended is probably to be expected. With the elbow flexed, the only muscle likely to be affected by alteration in the position of the shoulder joint is the biceps muscle. However, as the two heads of origin of this muscle arise close to the axis of the movement of the shoulder joint, the mechanical advantage of the muscle is probably not greatly altered.

On the other hand, when the elbow is extended and the lateral and medial rotators of the humerus play an active part in pronation and supination, the torque that these muscles can exert in humeral rotation will probably vary considerably in the different positions of the shoulder. Furthermore, there may also be different combinations of muscles acting in each of these positions. It is not possible to reach any conclusion on these points, for there appears to be considerable disagreement as to which muscles produce lateral and medial rotation of the humerus, and there is very little information on the effects of shoulder position on the action of these muscles.

Variation of maximum torque in the same shoulder-elbow positions. Although the methods were standardized as far as possible, there were still considerable variations in the recordings in the same shoulder-elbow-hand position. In other experiments on muscular exercise demanding a maximal effort, similar variations have been found (Duvall, Houtz & Hellebrandt, 1947; Wakim *et al.* 1950).

It might have been expected that there would be an improvement in successive runs in the same shoulder-elbow position indicating a 'training' effect. This was only shown statistically in two of the thirty-six runs. That it was not found more frequently may be due to the fact that either the physical condition of each of the subjects was good at the beginning of the experiments or that the experiments were of insufficient duration for a training effect to become obvious. Duvall *et al.* (1947) have similarly found no significant increase in muscle strength from a protracted repetition of a hand dynamometer test. Darling (1947) concludes that there is no improvement in muscular exercise unless the work requires a high degree of skill.

On the other hand, there may have been a 'training' effect which was masked by other variable factors, such as fatigue, which are acting in the reverse direction. Further, the work may have been performed with increasing efficiency; that is that there was a reduction in the energy required to develop a given force. There is, however, as yet no known method of measuring the energy cost with sufficient accuracy to detect possible changes in muscular exercise of short duration.

Although the experiments were designed to reduce fatigue to a minimum, it is impossible to say whether or not this was one of the factors responsible for the variations in readings. That immediate local fatigue was not important is indicated by the fact that the torques recorded in the first and second consecutive runs in each shoulder-elbow position were not significantly different.

In spite of efforts to control the posture of the subjects, it is possible that some of the differences observed in the same shoulder-elbow-hand positions on successive occasions may be due to slight changes in the position of the hand when gripping the handle of the dynamometer. These changes would alter the leverage and so lead to variations in the readings.

That the variations are greatest in those positions of the hand in which the torque exerted is smallest may be due to the fact that the muscles are being used in positions of the joints in which they are not normally called upon to contract. Under these circumstances, owing to the lack of general usage, neuromuscular co-ordination may not be fully developed.

A factor which was not controlled is the speed at which the isometric torque develops. Ralston, Polissar, Inman, Close & Feinstein (1949) conclude, from their work on isometric contraction in isolated human muscles, that a maximal effort leads

to the same or practically the same tension irrespective of the speed of onset. Whether this holds for intact muscle groups has still to be determined.

SUMMARY

1. The torque exerted in attempted pronation and supination of the right hand was studied in three subjects in different positions of the shoulder, elbow and hand. The torque was measured by means of an electrical strain-gauge dynamometer.

2. A linear relationship was found between hand position and the maximum torque developed in each of the shoulder-elbow positions. The torque of pronation increased as the position of the hand altered in a supination direction, and that of supination increased as the position of the hand altered in a pronation direction.

3. In the majority of shoulder-elbow positions, the average torque of pronation exceeded that of supination. This was particularly marked when the elbow was extended.

4. The factors responsible for the changes in the torque of pronation and supination in the different hand positions and shoulder-elbow positions have been discussed.

5. Considerable variations of the maximum torques were recorded in the same shoulder-elbow-hand position on successive occasions. The possible reasons for these variations have been considered.

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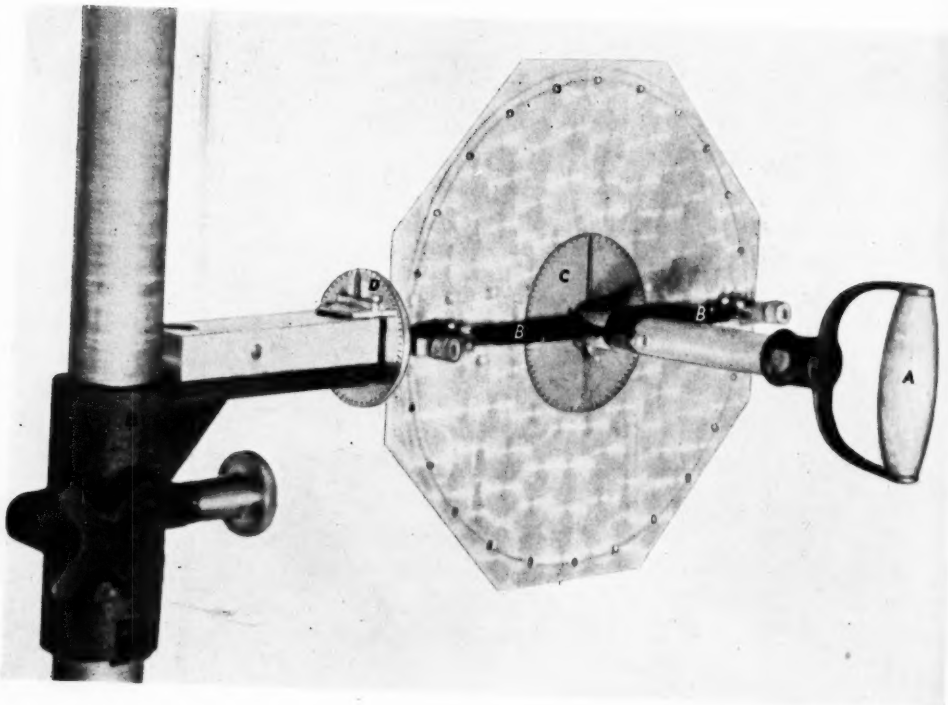

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EXPLANATION OF PLATE

Fig. 1. The strain-gauge dynamometer. *A*, hand-grip connected to metal spindle; *B*, tempered steel bar to which electrical strain gauges are fixed; *C*, scale indicating position of hand-grip; *D*, scale indicating tilt of dynamometer; *E*, adjustable support.

OBSERVATIONS ON THE INNERVATION OF THE CORNEA

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INTRODUCTION

A vast literature has accumulated on the innervation of the cornea, and there exist more than 300 publications dealing with various aspects of the subject.

Nevertheless, in 1910 Virchow, who summarized the literature up to that date, was struck by the fact that little attempt had been made to correlate the results of anatomical and physiological studies. The only serious attempt to synthesize morphological and functional observations was made by Ranvier as long ago as 1881, and since the time of Virchow the tendency towards specialization has become even more marked. That the need for correlation is still urgent may be instanced by the papers of Sédan and his colleagues (1947, 1948). Their clinical observations indicate that commonly accepted anatomical and physiological data cannot be satisfactorily harmonized and require both revision and amplification.

Clearly, before any further attempt can be made to integrate morphology and function, it is necessary to inquire critically into the present state of our knowledge concerning all aspects of corneal innervation.

The present conception of the morphology of the corneal nerves is based chiefly on the work of Attias (1912) and appears in current text-books of ophthalmology. Some authors, however, in particular Boeke & Heringa (1923), Boeke (1925, 1935) and Reiser (1935–6, 1936–7), have questioned some of Attias's findings. This lack of agreement on the precise anatomy of corneal innervation has wide implications. Thus it is not at present possible to compare normal and pathological anatomical observations in clinical medicine, even though nerve fibres can be observed in the normal living human cornea under the slit-lamp microscope. Prendergast (1940) suggests that diseases involving peripheral nerves, such as leprosy, can indeed be recognized at an early stage by such a comparison; but it is clear that his observations would be of greater significance if the anatomy of normal corneal innervation were known with certainty.

Since there is doubt concerning the anatomy of corneal innervation, the morphological approach cannot at present be used to settle physiological controversies, in particular those concerning the sensations which can be aroused from the cornea.

Until recently it was believed that pain was the only sensation which could be aroused from the cornea proper (von Frey, 1894, 1922; von Frey & Webels, 1922; von Frey & Strughold, 1926). Despite von Frey's assertions, Nagel (1895), Goldscheider & Brückner (1919), Franceschetti (1932*a*, *b*), Nafe & Wagoner (1937), Altschüler (1940), and a number of other authors, believe that touch as well as pain can be aroused from the normal cornea, but none of them has described any clear-cut anatomical arrangement which might lend weight to their subjective observations.

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Tower (1935, 1940, 1942) has shown by electrophysiological methods that the sensory receptive mechanism in the cornea consists of an aggregation of overlapping 'sensory units' which are physiologically independent. Weddell, Sinclair & Feindel (1948) have suggested that the quality of sensation (in particular pain) which can be aroused from tissues is connected in some way with the mode of distribution of the nerve terminals in the tissue.

There are a number of clinical observations that are related to the anatomical arrangement and to the functions of the corneal nerves. In cases of neuralgia treated by trigeminal tractotomy Sjöqvist (1938*a*, *b*), Rowbotham (1939), Grant, Groff & Lewy (1940), and Falconer (1949) found that pain sensibility was abolished and in some cases also the corneal reflex, but that stimulation of the cornea now gave rise to a sensation described as touch.

A number of clinical observations have been recorded which, at first sight, suggest that corneal nerves may have more than a purely sensory role. For instance, keratitis neuro-paralytica develops more commonly after section of the fifth nerve distal to the trigeminal ganglion than it does if the central root of the ganglion alone is divided, despite the fact that corneal sensation is entirely abolished in both cases (Magendie, 1824; Claude Bernard, 1858). There is as yet no experimental evidence that any sensory nerve fibres have 'trophic' functions (von Brücke, Hellauer & Umrath, 1949). Nevertheless, these clinical observations cannot at the moment be explained on purely anatomical and physiological grounds.

It is clear, therefore, that much basic work remains to be done before any attempt at a broad synthesis of knowledge is possible in the manner suggested by Virchow.

In this paper an attempt has been made to fill to some extent the gaps in our knowledge of the anatomy of corneal innervation, in such a way that an eventual integration of morphological and functional observations may be possible.

LITERATURE

Hoyer (1873), Ranvier (1881) and Virchow (1910) surveyed and summarized the literature up to the date of their publications. We shall, therefore, only refer to selected works which appeared before 1910, to indicate the development of thought on the nature of corneal innervation. We shall review all the publications known to us which have appeared since 1910.

For 20 years following the work of Schlemm (1831), who demonstrated, by ordinary dissecting methods, nerve fibres passing from the sclera as far as the corneo-scleral junction in man, stags and oxen, there was much controversy as to whether the cornea proper contained any nerve fibres whatever. Arnold (1832), Hueck (1839), and Beck (1847) held that the cornea was devoid of nerves, whereas Bochdalek (1837, 1839), working on corneas of large mammals and man, and Pappenheim (1839, 1840), working on the cornea of the ox and the stone eagle, held that the cornea proper was innervated. They noted the opaque whitish nerve fibres advancing from the sclera towards the corneal limbus described by Schlemm, but were able to demonstrate in addition fine translucent flattened threads arising from the nerve fibres and advancing for some millimetres into the substantia propria of the cornea.

The next phase of activity resulted from the development of histological methods.

Purkinje (1845), using corneas from different mammals, Koelliker (1848), using corneas from rabbits and frogs, Luschka (1850), using rabbits' corneas, Kühne (1862), using frogs' corneas, and Ciaccio (1863, 1881), using corneas from sparrows, eels, frogs, mice and man, found that after immersion of the tissue in dilute acetic acid they were able to see under the microscope nerve fibres in the substantia propria. They all gave good and more or less parallel descriptions of the arrangement of the corneal nerve fibres, and discussed the possibility of the axons ending in the form of a 'terminal net'.

His (1863) also studied the innervation of the cornea, using simple histological methods. He was in substantial agreement with his contemporaries concerning most points, but differed from them in that he believed the cornea to contain many nerve ganglion cells.

Kühne even discussed the relation of the nerve fibres to the cellular elements of the cornea, believing that they entered them, and he thereby started a controversy which has yet to be satisfactorily resolved. Kühne also believed that some of the corneal nerve fibres and cellular elements had a motor function and that they were in some way responsible for maintaining the translucency of the tissue. Lightbody (1867), in addition to publishing an excellent comparative anatomical study on vertebrate corneal innervation, also held that 'trophic' nerves entered the cornea from the region of the limbus where the capillary loops terminate. He also believed that the cornea contains many nerve ganglion cells.

Hoyer (1866), using impregnation methods involving silver nitrate and chromic acid, claimed that he was able to trace some nerve fibres through Bowman's membrane (anterior elastic lamina), on the surface of which they ramified, but was unable to trace any nerves beyond the membrane into the epithelium itself. Hoyer used corneas from newts, frogs, fish, birds, guinea-pigs, rabbits and man, but mentioned no major species difference in their mode of innervation.

The next step forward was taken by Cohnheim (1866, 1867), who introduced the gold chloride impregnation technique, and in a classical paper demonstrated that there are nerve fibres within the corneal epithelium in birds, frogs, rabbits and guinea-pigs. Despite Cohnheim's convincing drawings, however, Brand (1888) disputed his findings, maintaining that what Cohnheim described as nerve fibres were in fact precipitates of gold on the walls of the epithelial cells. He asserted his belief in the truth of Hoyer's statement that the corneal nerve fibres all terminate beneath the epithelium after penetrating Bowman's membrane.

As might have been expected, a large number of papers on corneal innervation followed the introduction of the gold chloride method. The majority of them were collected together and well reviewed by Ranvier. Unfortunately, however, the terminology used by the individual authors varies so widely that a critical assessment of their observations is not possible. Papers by Koelliker (1866), Engelmann (1867), Moseley (1871), Ladowsky (1872), Klein (1871, 1880), Hoyer (1873), Waldeyer & Yzquierdo (1880), and Ranvier (1881), are probably the most important contributions which appeared during this period. They studied variously corneas from mammals, birds, reptiles, amphibia and fish.

Arnstein (1887) and Dogiel (1890) took advantage of the work of Ehrlich (1886) and introduced the technique of methylene-blue staining of corneal nerve fibres.

Arnstein studied corneas from birds and amphibia. Dogiel (1891) made some particularly interesting observations on what he believed to be complicated terminations of nerve fibres in corneas taken from monkeys and from human subjects. He also demonstrated the presence of Krause end bulbs in the conjunctiva in these species.

Crevatin (1903), Bielschowsky & Pollack (1904) and Cajal (1909), using silver methods, studied corneas from mammals, birds and reptiles. Crevatin described the manner in which the nerve fibres ramify in the epithelium. Bielschowsky & Pollack noted particularly that many of the nerve bundles were flattened antero-posteriorly and called them nerve bands. Cajal, describing the innervation of the mammalian cornea, noted the absence of myelin sheaths and gave a good description of beaded axons terminating between the cells in all layers of the epithelium. Curiously enough, however, Cajal believed that there were no Schwann elements in any part of the cornea.

The next, or consolidation, period is notable for the papers of Virchow (1910), Attias (1912) and Agababow (1912). These authors mainly used methylene blue. They not only summarized the observations which had already been made (chiefly on animals) concerning corneal innervation, but themselves studied the human cornea in some detail. They were, however, chiefly concerned with the general pattern of innervation and the establishment of an agreed nomenclature, rather than with finer morphological details such as the exact position and mode of termination of the corneal nerve fibres, although they discussed these matters at some length. The paper of Attias is particularly notable for its solidity and for the fact that it has remained the standard anatomical reference on the innervation of the cornea up to the present day.

During this period, Nageotte (1911 *a–c*) published a series of papers dealing with the Schwann elements in relation to peripheral nerves, using a special technique developed for the purpose. In one of these papers (1911 *c*) he refers to the corneal nerve fibres in the rabbit. The only other papers dealing specifically with corneal Schwann elements appeared much later (Nageotte & Guyon, 1926; Nageotte, 1932; Boeke, 1935).

After 1912 no important papers specifically related to corneal innervation appeared until 1923, when the most recent phase of activity opened. It is concerned chiefly with the finer details of the innervation of the cornea. In 1923 Boeke & Heringa and in 1925 and 1935, Boeke published papers on the innervation of corneas from birds, frogs and macaque monkeys. They used silver and gold chloride impregnation methods to demonstrate the nerve fibres, and claimed that the axis cylinders eventually terminated within the cytoplasm of either the 'corneal corpuscles' or the epithelial cells. They also stated that a few corneal nerve fibres ended in an organized and complicated manner somewhat resembling a mode of termination described by Dogiel in 1891. Boeke differentiated between somatic and autonomic nerve fibres on the grounds that the former were thicker than the latter.

Eisler (1930), writing in the Schieck and Brückner *Handbook of Ophthalmology*, gave a description of corneal innervation based on the work of Attias, but, in addition, claimed that the nerve fibres terminated within the corneal cells in accordance with the views of Boeke. Nakajima (1930), using methylene blue in albino rabbits and pathological human eyes, also gave a description of the innervation of the cornea and in the main confirmed the findings of Attias, but did not refer to the work of Boeke.

Yokomatsu (1931), quoted by Reiser (1935–6), used a 'vital staining method' and claimed that the cornea contains a large number of different organized terminals.

Egorow (1934), using methylene blue, stained the corneal nerves in guinea-pigs and also claimed to have demonstrated many types of complicated terminals in the substantia propria and also organized nerve end-organs in the epithelium. In 1934 Ernyei, using osmic acid and silver methods, searched for myelinated nerve fibres and ganglion cells in the cornea of the rabbit. He examined many thousands of sections with negative results. Goto-Yotaro (1935), quoted by Reiser (1935–6), like Yokomatsu, used a 'vital' staining method and likewise claimed that the cornea contains many different types of organized terminals.

Reiser (1935–6, 1936–7), using silver methods, claimed that corneal nerve fibres ended in a 'terminal reticulum'. He also claimed that the innervation of the cornea in pigs and rabbits was very similar to that in man. He believed, like Stöhr (summarized in Stöhr, 1941), that the neurone theory was untrue, for he stated that he had evidence that peripheral nerve terminals are arranged in the form of a protoplasmic continuum. Lauber (1936), writing in von Möllendorff's *Handbuch*, gave a description of the innervation of the cornea based on the work of Attias (1912), Knüsel & Vonwiller (1922) and Knüsel (1923). The illustrations, which were furnished by Kolmer, are particularly good. Borri (1939), using silver methods combined with a vascular injection technique, noted the close relation between nerve fibres and blood vessels at the limbus of the rat's cornea, and believed that this might have a functional significance related to the secretion of the aqueous humour. Martinez (1940), using silver methods in human corneas, came to the conclusion that the nerves do not ramify within the syncytium formed by the corneal corpuscles, as claimed by Kühne and Boeke, but rather proceed along the surface of the cells. On the other hand, he claims to have seen nerve fibres forming protoplasmically continuous nets, and thus doubted whether the neurone doctrine could be taken quite literally.

Peris (1947, 1948), also using silver methods, has examined corneas from the following animals: bulls, sheep, rabbits, pigs, cats, chickens, frogs (*Rana esculenta*), snakes (*Tropinodotus natrix*) and man (foetal, prepubertal and adult specimens). He notes that his observations have revealed a fundamental similarity in the morphological arrangement of the corneal nerves in all the specimens he has examined, and that there are only minor species differences. His observations are in agreement with those of Martinez in so far as both authors deny that nerve fibres terminate within the cytoplasm of any of the cellular elements of the cornea. However, whilst Martinez is somewhat non-committal on the existence of nerve fibres in the epithelium, Peris is more definite and states that he has never seen nerve fibres within the corneal epithelium and does not believe in their existence.

Rodger (1950) investigated the innervation of the rabbit's cornea, using gold impregnation and methylene-blue staining. He observes that there are two types of nerve fibres in multifibre bundles; large beaded ones with fine dendrite links, and a second in which the beads are 'masked'. The fibres in which the beads are 'masked' he believes, on morphological grounds, to be autonomic.

In conclusion we shall review the work done on the innervation of the normal intact human cornea by workers using the slit-lamp and corneal microscopes. The first observations on the subject were made by Wolff in 1912. Erggelet (1914) merely confirmed Wolff's findings, but added nothing new. In 1919, Koeppe gave a fairly full account of the innervation of the cornea, using this method, and claimed to have

confirmed the findings of Attias. In 1922, Knüsel & Vonwiller and in 1923, Knüsel combined methylene-blue staining and the slit-lamp technique both in man and the rabbit and again claimed to confirm the findings of Attias in man. They did, however, note that there was a species difference in the pattern of corneal innervation without defining the differences clearly. So far, no facts concerning the neuro-anatomy of the normal cornea have been described by this method which are at variance with the work of Attias.

In summary, then, it is clear that the descriptive anatomy of corneal innervation given by Attias is still assumed in general to be correct and to apply, with minor differences, to all vertebrate species. There is no agreement, however, as to the manner in which the corneal nerves terminate.

MATERIAL AND METHODS

We have examined corneas from the following vertebrates: the dogfish (*Scyllium canicula*—10 animals), the frog (*Rana temporaria*—15 animals), the white rat (10 animals), the guinea-pig (many varieties—25 animals), the rabbit (many varieties—104 animals), the monkey (*Macaca mulatta*—4 animals), and man (5 subjects). The details of the special techniques that we have employed in this investigation have already been published (Weddell & Zander, 1950). They include:

(1) Direct observation of untreated corneas *in situ*, using the slit-lamp and corneal microscopes. (54 examinations.)

(2) The immediate examination of whole preparations, or razor slices from the untreated corneas of anaesthetized animals, under the phase-contrast microscope. (52 specimens.)

(3) Vital staining methods employing methylene blue. (211 specimens.)

(4) Impregnation methods employing gold and silver salts. (75 specimens.)

(5) Myelin stains. (4 specimens.)

(6) Nageotte's method for demonstrating Schwann cells. (4 specimens.)

(7) Methods for demonstrating connective tissue elements following silver impregnation (see Holmes & Young, 1942).

NOMENCLATURE

Since there is no agreement as to the precise meaning of certain terms in common use, we felt that it was necessary to state exactly what we wish to imply when, for the sake of necessity or continuity, we employ terms which at present lack definition.

(1) The term nerve bundle refers to an aggregation of either myelinated or non-myelinated nerve fibres running in close proximity to one another for some distance. The individual axis cylinders within the nerve bundle may be running either towards or away from the central nervous system, and may or may not divide when traced in continuity along the bundle. If a nerve bundle is surrounded by a demonstrable epineurial connective tissue sheath, this fact will be specifically referred to in the course of our description.

(2) The terms branch or division, when used in relation to nerve bundles, are used in their colloquial sense; that is, it is implied that a proportion of the nerve fibres from the bundle pass into each branch or division. If axon multiplication takes place at or in a branch or division, this will be specifically referred to.

(3) The term plexiform arrangement is likewise used in its colloquial sense, but also specifically implies that axons derived from different sources do not fuse with one another when entering into this relationship.

(4) The term net formation implies the fusion in protoplasmic continuity of one or more of the axis cylinders derived by branching from a single parent axon.

(5) The term Schwann element implies the presence of one of the following: Schwann nucleus, Schwann cell cytoplasm, Schwann cell membrane, Schwann sheath (neurilemma) or endoneurium. When we can be more specific we shall be, and then the terms we use will have the meaning assigned to them by Holmes & Young (1942).

OBSERVATIONS

(1) *The arrangement of the nerve bundles supplying the cornea in the rabbit*

(*a*) *Origin of nerve bundles*

Bundles from the ciliary nerves pass forward as far as the ciliary bodies. The majority advance towards the limbus between the sclera and the choroid in the stratum perichoroideum; the remainder in the substance of the sclera. The corneal nerve fibres diverge from those passing to the ciliary body and iris just proximal to the corneo-scleral junction. We have never seen bundles of nerve fibres approaching the cornea from other sources.

The nerve bundles entering the cornea are between 61 and 68 in number, of which about 30 contain between 15 and 30 axons, the remainder containing less than 15 (Table 1). For the purpose of description they can be said to lie in three planes: the

Table 1. *Number of nerve bundles at corneo-scleral junction*

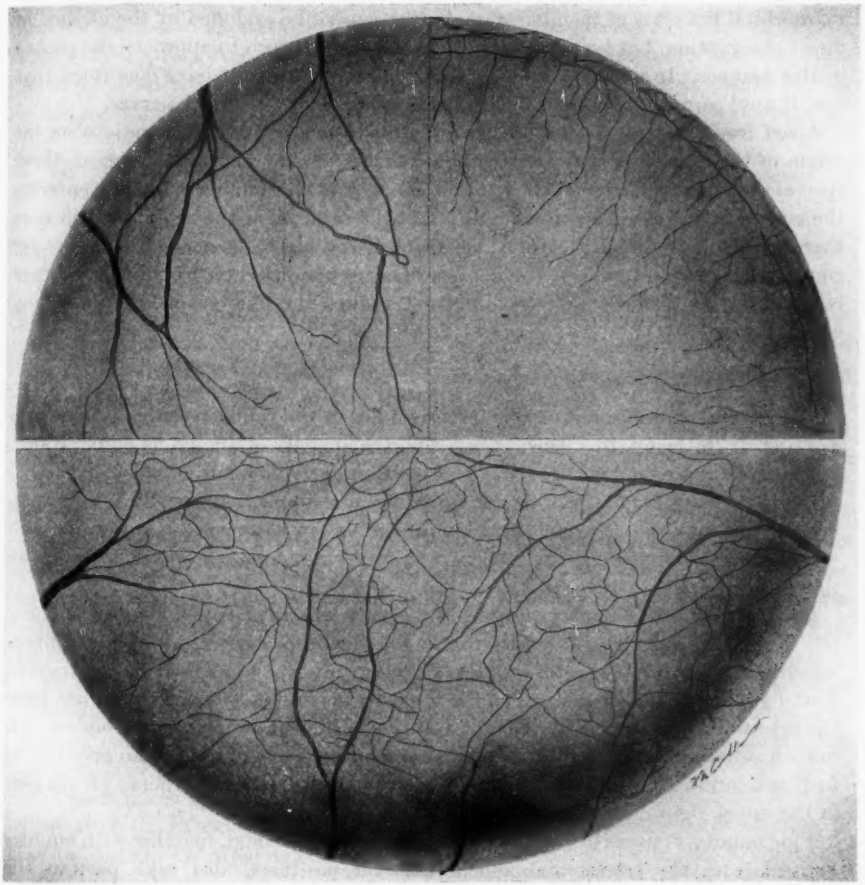
No.	Frog	Rabbit	Macaque monkey	Man
1	24	66 (32)	72 (37)	82
2	28	64 (31)	67 (33)	84
3	22	61 (34)	68 (32)	76
4	23	68 (29)	—	78
5	29	62 (33)	—	—

The figures in brackets indicate the number of bundles containing fifteen or more axons.

scleral, the episcleral and the conjunctival. The nerve bundles entering in the scleral plane are arranged in many different ways as illustrated in Text-fig. 1 (left upper quadrant). The bundles entering from the episcleral position are derived as follows: a proportion of nerve bundles leaves the sclera to pass directly into an episcleral position, where they join with medium-sized bundles derived from those which have entered the cornea in the deeper scleral plane; together they give rise to an episcleral pericorneal plexus (plexus paramarginalis of Attias; plexus annularis of Ranvier) (Text-fig. 1, right upper quadrant; Pl. 1, fig. 1). From this ring-like scaffold of nerve bundles, which incidentally is best demonstrated by the method of intravenous injection of methylene blue, further nerve bundles arise and pass into the most superficial layers of the substantia propria of the cornea.

The subconjunctival bundles are small and proceed towards the limbus from the deepest subepithelial layer of the conjunctiva, most of them being derived from the episcleral pericorneal plexus, but a few coming directly from those proceeding to

the limbus in the scleral position. The subconjunctival bundles can readily be distinguished from those forming the episcleral annular plexus, for they are in close



Text-fig. 1. This diagram illustrates the arrangement and behaviour of the various nerve bundles which enter the periphery of the cornea in different planes. The drawing is a composite one based upon observations from a large number of methylene-blue stained rabbits' corneas. The form and behaviour of nerve bundles entering the cornea from the scleral position is shown in the upper left quadrant. The form and behaviour of nerve bundles entering the cornea from the episcleral and subconjunctival positions are illustrated in the upper right quadrant. In the lower half of the drawing is shown the manner in which the plexiform pattern of nerve fibres arises from the nerve bundles. It also shows the passage of nerve bundles across the centre of the cornea from limbus to limbus, and the fact that not all the nerve bundles in the cornea are radially disposed.

relation to blood vessels and have a similar sinuous course (Pl. 1, figs. 2, 3). The sinuosity is not a fixation artefact, for vessels and nerves can be seen under the corneal microscope to take such a course in the living eye. The branches which leave the

subconjunctival bundles pass both into the superficial layers of the substantia propria and into the epithelium of the cornea. The possibility that a few of the nerve bundles which enter the cornea from the subconjunctival position are derived from extraorbital branches of the fifth cranial nerve cannot be excluded by the method of direct observation, but no large bundles have ever been seen to approach the cornea in this manner. In addition, recent degeneration experiments have confirmed that few, if any, corneal nerves are derived from other than the ciliary nerves.

Apart from those in the rabbit, we have made comparative observations on the origin of the corneal nerves only in the macaque monkey and man. In both these species the general plan is similar to that in the rabbit. The number of bundles entering the cornea in the macaque monkey is about the same as in the rabbit, but in man there are about 70–80 bundles, of which about 40 contain 15–20 axons, the remainder containing less than 15 fibres. The number of nerve bundles entering the frog's cornea is about 15–20 (Table 1). We have not determined the number of bundles in any other species.

(*b*) *Arrangement of nerve bundles*

The general arrangement of the nerve bundles in the rabbit's cornea is shown in Text-fig. 1. Although this figure is based on the analysis of numerous preparations, each bundle represents in form, size and position one which has actually been seen. Although certain bundles can be seen dividing in a simple manner (Pl. 1, figs. 4, 5), it is not profitable to segregate these bundles into a few clear-cut categories, for each would merge too freely into the other. There are certain points, however, to which we should like to draw attention.

Most of the large bundles entering the cornea are arranged in a radial manner (Pl. 2, fig. 19), and ultimately give rise to the plexiform aggregation of nerve fibres which pervades the substantia propria (Pl. 2, fig. 16). The top left-hand quadrant in Text-fig. 1 shows the form taken by the large nerve bundles entering the cornea from the sclera. They enter the middle third of the substantia propria of the cornea and branch, subdivide and join in the manner indicated. These bundles also give rise to further bundles which take part in the formation of the pericorneal nerve plexus seen in the upper right quadrant in Text-fig. 1.

Fine bundles of nerve fibres leave the pericorneal plexus and, together with bundles derived from the scleral and subconjunctival positions, also take part in the formation of the plexiform aggregations pervading the substantia propria.

In the lower half of Text-fig. 1 and Pl. 20, figs. 2, 21, are shown bundles of nerve fibres which join with one another, but which spring from widely separated positions on the periphery of the cornea.

The smaller nerve bundles all combine to form the plexiform aggregations of nerve fibres disposed in up to five layers (plexus fundamentalis of Ranvier, or Hauptplexus of Attias) throughout the substantia propria of the cornea from Descemet's (posterior elastic lamina) to Bowman's membrane (Pl. 2, figs. 16–18). The density of the plexiformly arranged nerve bundles is greater towards the surface of the substantia propria.

In the macaque monkey and in man the number of radially arranged nerve bundles which join to form loops is greater towards the centre and less towards the periphery

of the cornea than in the case of the rabbit. In the rat and in the guinea-pig the plexiformly arranged nerve fibres do not form so fine a meshwork as they do in the rabbit, for the number of layers in which they are disposed is reduced to two or three, and the number of axons in each aggregation is greater. In the frog and dogfish the meshwork appears even less dense, and it is difficult to define more than one main layer, situated closer to Descemet's membrane than in the case of the mammals which we have examined. No other important species differences were noted.

(2) *The form and behaviour of individual nerve fibres in the substantia propria of the rabbit's cornea*

(*a*) *Behaviour of axons in large nerve bundles*

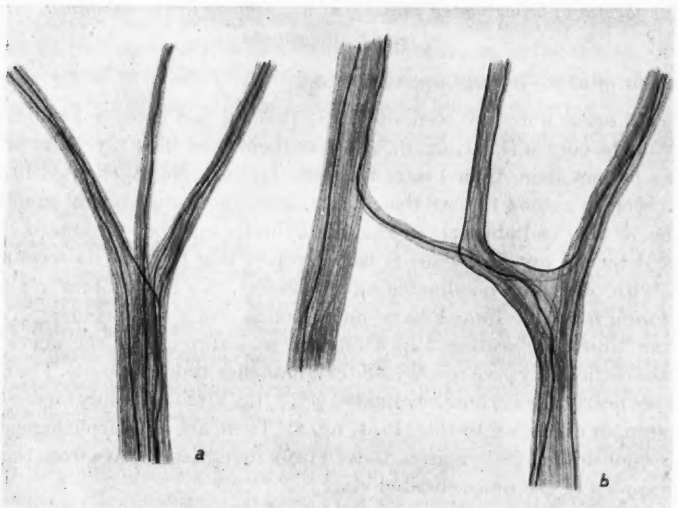
Myelinated nerve fibres are seen only in the scleral and episcleral bundles at the periphery of the cornea (Pl. 1, fig. 6). Most of them shed their myelin sheaths after proceeding for not more than 1 mm. from the limbus. No myelinated fibres have ever been seen to extend farther than 2 mm. from the corneo-scleral junction. The myelin sheath may end abruptly or the axis cylinder may be surrounded by one or two short detached myelin segments before proceeding towards its termination in company with other non-myelinated axis cylinders. No nerve fibres in the cornea beyond 2 mm. from the limbus have nodal points along their course. Myelinated nerve fibres which are scattered in a random way throughout the nerve bundles entering the cornea may give rise to collateral branches at nodal points. The collateral branches are nearly always non-myelinated (Pl. 1, fig. 7); in fact, only on one occasion have we seen an exception to this (Pl. 1, fig. 8). There are no morphological criteria which distinguish the axis cylinders derived from myelinated fibres from those which enter the cornea in the unmyelinated state.

At the limbus when a large nerve bundle divides, axon multiplication is rare (Text-fig. 2*a*, *b*). In bundles which divide closer to the centre of the cornea, axis cylinder multiplication takes place more often, but at the same time individual axons are seen to be pursuing more complicated courses. In Text-fig. 3*a–d* are drawings of the course taken by different axons in such positions. In Table 2 figures are given illustrating the extent of axon multiplication, having regard, in so far as possible, to the difficulties illustrated in Text-fig. 3. That the course taken by a certain number of axons was nevertheless recurrent cannot be excluded (Pl. 1, fig. 9), for detailed examinations were made only in the vicinity of the bifurcations. Degeneration experiments suggest that the counts are in fact of some quantitative value, for, although the course of some axons is recurrent in the manner suggested, the number that would have been missed by careful examination in the region of the division is very small.

In nerve bundles which pass across the centre of the cornea, such as those seen in Text-fig. 1, and Pl. 2, figs. 20, 21, some axons extend from one limbus through the centre of the cornea towards the opposite limbus, but so far it has not been possible to trace an individual axon further than three-quarters of the way across the cornea.

Single axons within nerve bundles entering the cornea pursue varying and complicated courses and give rise to numerous daughter axons. When traced from their entry towards their terminations, individual axons have never been seen to give rise

to more than two daughter axons at any point of division. Axon division is occasionally seen in the course of a nerve bundle, in which case one of the daughter axons is usually of smaller diameter than the other, which passes on its way little diminished in size (Pl. 1, figs. 7, 8). In rare instances, however, both parent and daughter axons appear to be of the same diameter. One of the daughter axons always springs from the parent axon approximately at a right angle (Pl. 1, figs. 10, 12), then abruptly changes its course to that of the direction of the main nerve bundle itself.



Text-fig. 2 (*a–b*). These drawings are from methylene blue-stained rabbits' corneas. They summarize the actual course and behaviour of individual axons which have been seen at points of division of nerve bundles at the limbus. Axon multiplication is rare.

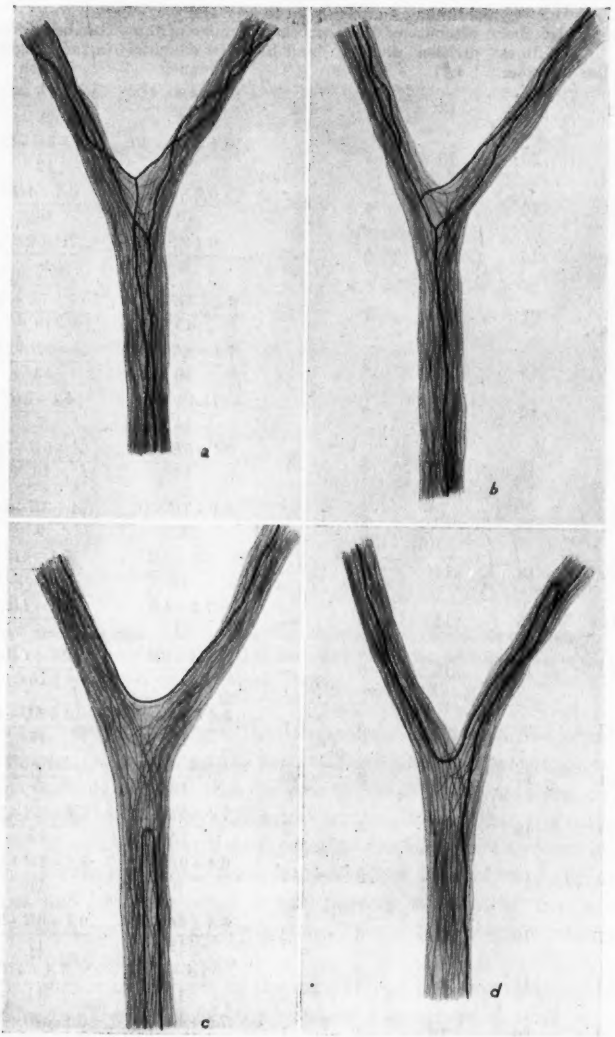
It is, of course, possible to argue that these appearances are the result of fusion taking place between daughter axons derived from different parent axons. In cases in which it was possible to determine the course and destination of the axis cylinders concerned, two axons were always traced to terminations within the cornea and the remaining axon to a single parent stem entering the cornea. In recent experiments in which corneas were examined some 3 weeks after a number of the nerve fibres supplying them had been destroyed it was possible to confirm these observations more easily. In no cornea has fusion ever been seen to take place between collaterals derived from different parent axons.

In any nerve bundle in the cornea the diameter of the axis cylinders apparently varies from 1 to 5μ. The axons of larger diameter are those at some distance from their terminations, and, when traced peripherally, they progressively decrease in diameter. At points of bifurcation one daughter axon may be of greater diameter than the other (Pl. 1, fig. 11). However, in every case which we have analysed, the axon of smaller diameter gave rise to fewer branches and terminated before the

Table 2

Rabbit no.	Bundles	Number of fibres in stem	Number of fibres continuing without division (*A*)	Number of fibres dividing (*B*)	Number of fibres passing from one daughter axon to another branch (*C*)	Number of fibres in 1st daughter branch	Number of fibres in 2nd daughter branch	Total fibres in daughter branches
1	1	17	13	4	1	$\frac{9A+4B+1C}{14}$	$\frac{4A+4B+1C}{9}$	23
	2	20	15	5	2	$\frac{10A+5B+2C}{17}$	$\frac{5A+5B+2C}{12}$	29
	3	16	12	4	—	$\frac{6A+4B}{10}$	$\frac{6A+4B}{10}$	20
	4	15	13	2	—	$\frac{6A+2B}{8}$	$\frac{7A+2B}{9}$	17
2	5	21	14	7	2	$\frac{4A+7B+2C}{13}$	$\frac{10A+7B+2C}{19}$	32
	6	19	11	8	1	$\frac{7A+8B+1C}{16}$	$\frac{4A+8B+1C}{13}$	29
	7	18	15	3	—	$\frac{11A+3B}{14}$	$\frac{4A+3B}{7}$	21
	8	19	14	5	3	$\frac{6A+5B+3C}{14}$	$\frac{8A+5B+3C}{16}$	30
3	9	16	13	3	1	$\frac{8A+3B+1C}{12}$	$\frac{5A+3B+1C}{9}$	21
	10	18	14	4	—	$\frac{9A+4B}{13}$	$\frac{5A+4B}{9}$	22
	11	15	11	4	—	$\frac{7A+4B}{11}$	$\frac{4A+4B}{8}$	19
	12	19	13	6	—	$\frac{4A+6B}{10}$	$\frac{9A+6B}{15}$	25
4	13	20	15	5	1	$\frac{5A+5B+1C}{11}$	$\frac{10A+5B+1C}{16}$	27
	14	17	14	3	—	$\frac{9A+3B}{12}$	$\frac{5A+3B}{8}$	20
	15	18	12	6	2	$\frac{7A+6B+2C}{15}$	$\frac{5A+6B+2C}{13}$	28
	16	16	14	2	1	$\frac{6A+2B+1C}{9}$	$\frac{8A+2B+1C}{11}$	20
5	17	19	13	6	2	$\frac{4A+6B+2C}{12}$	$\frac{9A+6B+2C}{17}$	29
	18	18	14	4	—	$\frac{7A+4B}{11}$	$\frac{7A+4B}{11}$	22
	19	20	16	4	1	$\frac{7A+4B+1C}{12}$	$\frac{9A+4B+1C}{14}$	26
	20	17	11	6	—	$\frac{4A+6B}{10}$	$\frac{7A+6B}{13}$	23

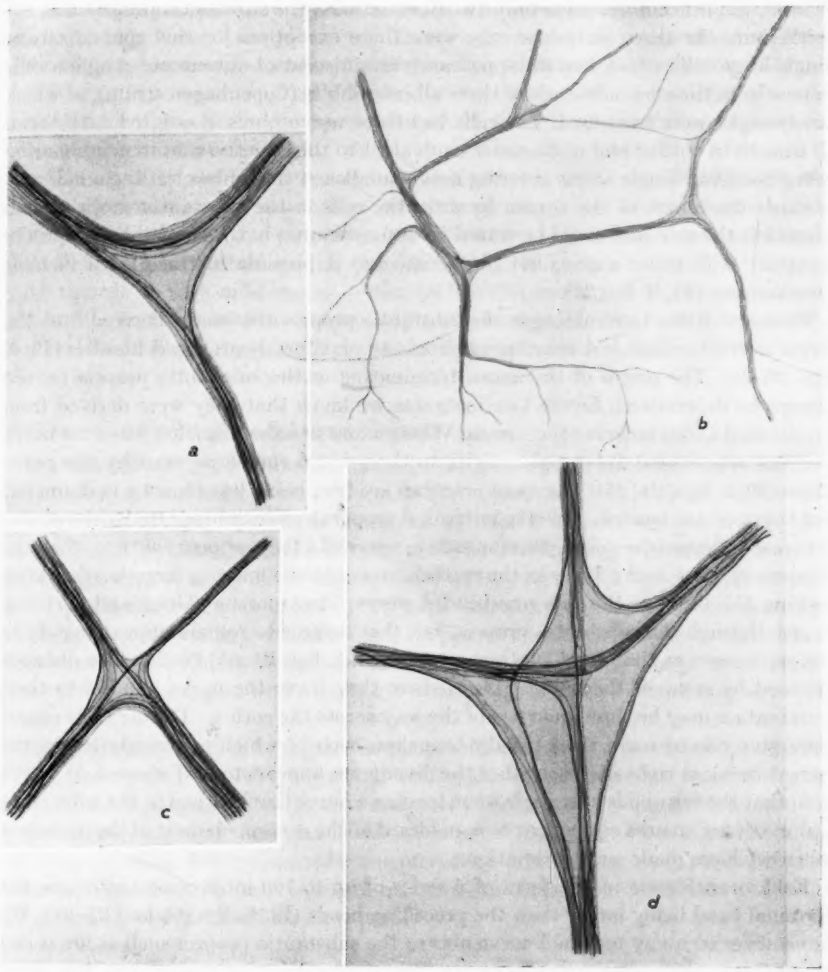
larger axon. In methylene blue-stained specimens, swellings often appear at the position of axon division (Pl. 1, figs. 12, 13). In some instances the swelling is large, being up to five times the diameter of the axons. In every case all three axis cylinders appear to enter and become continuous with the swelling (Pl. 1, fig. 13).



Text-fig. 3 (*a–d*). These drawings are from methylene blue-stained rabbits' corneas. They summarize the actual course and behaviour of individual axons at points of division of nerve bundles in the middle third of the cornea. See table 2 for quantitative data.

(*b*) *Behaviour of axons in the plexiformly arranged nerve bundles*

Axons in the plexiformly arranged nerve bundles usually bifurcate at the angles of the meshwork to which they give rise, but not invariably. The behaviour of those which do not divide is shown in Text-fig. 4 *a–d*. Axon multiplication, however, is rare other than at the points of division of nerve bundles. The axons forming these patterns lie between the laminae of the substantia propria and are flattened antero-



Text-fig. 4 (*a–d*). These drawings are from methylene blue-stained rabbits' corneas. They summarize the actual course and behaviour of individual axons in the plexiform arrangement of nerve fibres in the substantia propria. The prominent axons displayed are the rarer ones which did not divide.

posteriorly, the nerve fibres tending to lie side by side rather than in cable form. The nerve fibres connecting them pass steeply, sometimes almost vertically, from one layer to the next and are also flattened, but in a vertical plane which makes their arrangement difficult to resolve under the microscope (Pl. 1, figs. 14, 15).

(*c*) *Behaviour of axons terminating in the substantia propria*

With the methods at our disposal we can distinguish corneal nerve fibres from one another only by virtue of the fact that they have different destinations. In general, the nerve fibres have only two destinations: the substantia propria and the epithelium. In three instances only were there exceptions to this generalization. Single large cells which had an appearance reminiscent of autonomic ganglion cells were seen in the cornea of each of three albino rabbits (Copenhagen strain), of which ninety-eight were examined. The cells had three nerve fibres associated with them, all smooth in outline and of diameter equivalent to the largest axons in neighbouring nerve bundles. Single axons entering nerve bundles at the limbus left them half way towards the centre of the cornea to enter the cells in the substantia propria. Two axons left the cells and could be traced for some distance in the substantia propria in company with other axons; but in no case was it possible to trace them to their terminations (Pl. 3, fig. 22).

The nerve fibres terminating in the substantia propria are usually derived from the larger nerve bundles, but may spring from the plexiformly arranged bundles (Pl. 3, figs. 23–25). The origin of the axons terminating in the substantia propria cannot always be determined, but in two instances we know that they were derived from myelinated axons entering the cornea. Whole axons or axon branches leave the nerve bundles approximately at right angles to the general course pursued by the nerve fibres (Pl. 3, figs. 24, 25). The axon branches are fine, being less than 1 μ in diameter, but they are not beaded. Even in untreated preparations examined under the phase-contrast microscope some 60 min. after removal, they appear as fine threads, whereas the fine nerve fibres in the epithelium contain numerous large beads. After leaving the bundles the fine non-beaded nerve fibres pursue a long and tortuous course through the substantia propria, but they appear to remain approximately in the same layer as that in which they entered (Pl. 3, figs. 24 and 26–28). The distance pursued by some of these fibres from where they leave the nerve bundles to their termination may be three-quarters of the way across the cornea. During their course they give rise to more than twenty branches, each of which commonly leaves the parent trunk at right angles, so that the divergence appears to be T-shaped. We have seen that the terminals of a single axon leaving a nerve trunk to end in the substantia propria cover an area equivalent to a quadrant of the cornea, on most of the occasions when we have made such estimations.

Each branch ends in the form of a series of up to 100 interconnected beads, the terminal bead being larger than the preceding beads (Pl. 3, figs. 23 and 25–27). We have never seen any terminal nerve nets in the substantia propria such as are sometimes seen in the epithelium. The axons change their direction many times, but never re-enter nerve bundles once they have left them, even when by doing so they would reach their destination more directly.

A number of axons give rise to daughter axons at intervals along their course in

a bundle. Each daughter axon behaves and terminates in the same way, but is situated in a different layer of the substantia propria. Nerve fibres and terminals of this kind are seen in every layer of the substantia propria from Bowman's to Descemet's membrane, but in the rabbit they are far more numerous towards the epithelium.

Occasionally in methylene blue preparations droplets of stain are seen lying amongst the polygonal cells lining Descemet's membrane, but they do not appear to be terminal axon beads, for when preparations of these cells are stripped away from the substantia propria they are found to be free from nerve fibres and terminals (Pl. 3, fig. 30). None of the other methods we have used to demonstrate nerve fibres and terminals has shown any to be situated in close relation to Descemet's membrane.

We have never seen any complicated or organized nerve terminations on the corneal side of the limbus in specimens which have been carefully prepared for microscopic examination.

It has not been possible to demonstrate any protoplasmic connexion between the cellular elements in the substantia propria and the axons under phase-contrast conditions. In thin (3–5μ) *silver impregnated* sections, however, it was exceedingly difficult to be quite certain on this point (Pl. 3, fig. 29).

As regards species differences, in the rabbit, the monkey and man the axons terminating in the substantia propria appear to be equally abundant. They are not so numerous in the guinea-pig and rat, and still less numerous in the dogfish and frog, where they are more deeply situated.

(*d*) *The arrangement of the Schwann elements*

The relation of the Schwann elements to the axis cylinders in the nerve bundles and smaller aggregations of nerve fibres in the substantia propria of the cornea and to the axons which leave them to terminate in beads is in sharp contrast. Schwann cell nuclei and cytoplasm can be demonstrated by overstaining with methylene blue (Pl. 4, figs. 31, 32) or by silver impregnation (Pl. 4, fig. 33). They can also be seen under phase-contrast conditions in fresh untreated specimens mounted in saline (Pl. 4, fig. 34). Whatever method is used, it appears that the axons lie on the surface of Schwann cell cytoplasm during their course along the nerve bundles and finer nerve fibre aggregations, and even for a short distance when proceeding as single axons.

The single axons which terminate in the substantia propria, however, proceed for a large part of their course lying naked among the fibrous elements which make up the substantia propria (Pl. 4, figs. 33 and 35–38), where they come in close relation to corneal corpuscles or their plasmatic processes (Pl. 3, fig. 29; Pl. 4, fig. 38). It has not been possible to demonstrate unequivocally the existence of perineurial or endoneurial connective tissue in relation to any of the aggregations of nerve fibres in the cornea by the methods we have used.

(3) *The innervation of the corneal epithelium in the rabbit*

Nerve fibres leave the plexiform arrangement and penetrate Bowman's membrane almost perpendicularly to reach the epithelium (Pl. 4, fig. 39; Pl. 5, figs. 42, 43). At the limbus a few fibres enter the corneal epithelium directly from the conjunctiva.

The axons which enter through Bowman's membrane appear to pass through definite apertures in an otherwise homogeneous substance. On entering the corneal epithelium from whatever position, the axons shed their Schwann cell companions and pursue their course entirely naked, lying in direct relation to the surfaces of the cells composing the epithelium.

After penetrating Bowman's membrane the naked axis cylinders often divide into a leash of as many as fifteen fibres, all of which in methylene blue-stained and gold-impregnated specimens are beaded (Pl. 4, figs. 39, 41; Pl. 5, figs. 42–47). Under phase-contrast conditions and after gold impregnation the nerve fibres are seen to run in between the cells of the basal layer of the epithelium and not in a plane between Bowman's membrane and the cells (Pl. 4, figs. 40, 41; Pl. 6, fig. 49). In the rabbit the leash of fibres is orientated radially, the fibres tending to advance towards the centre of the cornea, except at the limbus where they scatter in many directions (Pl. 2, fig. 17; Pl. 5, figs. 42, 43).

Many axons terminate in the plane of the basal layer of the epithelium (Pl. 6, fig. 50). Occasionally, in methylene blue-stained material, adjacent axis cylinders derived from a single axon penetrating Bowman's membrane appear to fuse with one another, forming a protoplasmatically continuous net, but this is uncommon (Pl. 6, fig. 55). Under phase-contrast conditions such appearances have been seen, but are even more uncommon than after methylene-blue staining (Pl. 6, fig. 52). The possibility that they are an optical illusion cannot be dismissed, for the only specimens which can be used for an assessment of this kind are thicker than is desirable on optical grounds. We have never seen fusion between branches derived from axons piercing Bowman's membrane separately. The majority of axons terminate freely without making interconnexions, but the plane in which they terminate varies slightly (Pl. 6, figs. 48, 57).

Arising from the fibres which pierce Bowman's membrane and from fibres of the leashes into which they divide, are further branches which pass into the superficial layers of the epithelium (Pl. 6, figs. 53–55), advancing between the cells. The fibres may pass vertically towards the surface and end in between the cells of the most superficial layer, or take a more oblique course between the epithelial cells. These fibres and their beads are in every way similar to those lying between the cells of the basal epithelial layer. As the fibres advance through the epithelium, they multiply by axon branching (Pl. 6, figs. 55–57). The more vertically disposed fibres are arranged in a tree-like manner and their branches terminate between the epithelial cells of all layers (Pl. 6, fig. 57); they are usually about twice the diameter of the more obliquely disposed fibres. The latter tend to arborize in a plane parallel to the surface of the cornea, and here again branches from a single axon may appear to fuse to form protoplasmically continuous nets which lie in a single plane. The 'nets' are polyhedral in form and are disposed between the cells of the epithelium. From the 'nets' arise terminal branches ending in adjacent layers between the epithelial cells.

We have referred to 'vertically' and 'obliquely' disposed fibres passing towards the surface of the epithelium. Between these two extremes lies a continuous spectrum of fibre diameters approaching the surface of the epithelium at varying degrees of obliquity.

The axons in the corneal epithelium pursue independent courses towards their

termination. Although they overlap with their neighbours, they do so in different planes, and there is no close interweaving of fine unmyelinated beaded axons such as is seen just beneath the epidermis in the skin (Pl. 6, figs. 48–51).

The axons connecting the beads in the epithelium, even when examined in the whole preparations under unfavourable optical conditions, appear to be less than 1μ in diameter, although there is a variation in the diameter between the fibres and along the course of a single fibre in the proportion of about one to three (Pl. 6, fig. 51). The variation in diameter is accounted for, in part, by the fact that the fibres become progressively, although irregularly, finer as they are traced towards their terminations.

Since, on optical grounds, it is not possible to make quantitative measurements of fibre diameters in whole preparations, we prepared frozen sections of methylene blue-stained corneal epithelium. The axons connecting the beads in these preparations also appear to be less than a micron in diameter, and are thus too fine to be measured accurately even under these conditions (Pl. 6, figs. 51, 56 and 57). In the best silver preparations the appearances are similar (Pl. 6, fig. 58). The beads are approximately two or three times the diameter of the connecting axons, and hence along the thicker fibres the beads appear larger and more prominent than along finer fibres (Pl. 6, fig. 51). The terminal bead is commonly somewhat larger than the other beads (Pl. 6, figs. 55–58). The distance between the beads does not vary much, being approximately 10μ. Under phase-contrast conditions the beads along the course of axons in the epithelium may appear smaller than after methylene-blue staining or gold impregnation (Pl. 6, figs. 49, 52 and 53). In preparations examined *immediately* after removal the beads are not more than half as large again as the connecting axon. In specimens kept for a longer period the beads appear similar to those described above.

Under phase-contrast conditions we have never seen any nerve terminals lying within the cytoplasm of an epithelial cell; neither were we able to demonstrate any basket-work of fine fibrils proceeding from terminal beads to envelop any cellular elements present.

In the guinea-pig an axon piercing Bowman's membrane gives rise to a leash of between six and ten daughter axons (Pl. 5, figs. 43, 44); in the macaque monkey and man between ten and twenty axons. In the frog an axon, after piercing Bowman's membrane gives rise to a stellate formation rather than a leash of daughter axons. In the guinea-pig the more vertically disposed axons appear in somewhat greater number than they do in the rabbit and in man.

DISCUSSION

In 1910, Virchow stated that the chief anatomical problems in relation to corneal innervation which required investigation were three in number: the structure of the axis cylinders and the tissues immediately surrounding them; the relation of the corneal nerves and their terminals to the tissues composing the cornea; and finally the significance of the plexiform arrangement of the axons in the cornea. In the literature published since then, the descriptive anatomy of the innervation of the cornea given by Attias in 1912 is in general accepted as correct, but there is no agreement as to the nature of corneal innervation.

Most of the literature on the innervation of the cornea is in German and some relevant publications are not available in this country. Moreover, no critical review of the literature has appeared since Virchow's time. For these reasons we shall discuss the controversies raised in the literature in some detail, reviewing them in the light of our own observations.

(1) *The distribution and termination of the corneal nerves*

We have shown by improved histological methods that there are direct pathways in the form of nerve bundles of varying sizes passing from one limbus to the other through the centre of the cornea, a fact disputed by Attias. Moreover, we have been able to trace single axons from one limbus three-quarters of the way through the centre of the cornea towards the opposite limbus before they were seen to terminate.

Apart from this, both Attias' and Knüsel's descriptions of the arrangement of the corneal nerves in the rabbit are in general similar to ours. In man, however, they were unable to observe any plexiformly arranged nerve fibres except in the periphery of the cornea, where they noted the existence of a 'plexus paramarginalis superficialis'. On the other hand, we have been able to demonstrate the existence of plexiformly arranged nerve fibres throughout the human cornea. Species differences between man and the rabbit may account in part for the findings of Attias and Knüsel. In man more nerve bundles proceed in a straight course almost to the centre of the cornea before terminating in branches which go to form the plexiformly arranged pattern of nerve fibres. The bundles give off branches on their course just as they do in the rabbit, but nevertheless contrive to give the appearance of a more extended radial formation.

Our findings in relation to the descriptive anatomy of the nerve bundles in man are in broad agreement with those of Reiser (1935–6), using silver methods, and those of Schornstein (1934) in pathological cases in man in which the corneal nerves were myelinated. In the frog we have confirmed the findings of Lipmann (1869), who demonstrated that the plexiform arrangement of nerve fibres was denser in the depths of the substantia propria rather than beneath Bowman's membrane—the reverse of the condition seen in man.

We have also demonstrated, by the examination of fresh untreated specimens under phase-contrast conditions, that nerve fibres terminate between the cells in all layers of the corneal epithelium, even between the cells of the most superficial layer, but that they do not terminate between Bowman's membrane and the cells of the basal layer of the epithelium. These facts have been much disputed in the past and no clear-cut decision on the subject emerges from a perusal of the literature.

It appears that only under phase-contrast conditions is it possible to examine the corneal epithelium in its unshrunken state, and that previous workers have been handicapped by having to use histological methods involving fixatives in which a variable degree of shrinkage is inevitable.

The corneal epithelium is innervated by beaded terminals disposed 'in depth' throughout its whole extent, unlike the epithelium of the skin in which the fine beaded nerve fibres appear to terminate chiefly by a process of interweaving with one another in a plane just beneath the basal layer of the epithelial cells.

Nerve terminations are seen throughout the whole extent of the substantia propria

in all the species which we examined. They take the form of a few small terminal beads arising from single fine nerve fibres, smooth in contour, which have pursued an exceedingly complex course through the substantia propria. They are far less numerous (in proportion of about 1 to 50 in the rabbit) than those seen in the epithelium, and the beaded terminal is characteristically short. There is no doubt that the staining was adequate in our preparations, for their appearance is similar under phase-contrast conditions; moreover, terminals of this type can be demonstrated by silver and gold impregnation techniques.

As long ago as 1867, Engelmann described nerves ending freely in the substantia propria. Attias, however, describes the presence of only a few fine nerve fibres ending in small terminal expansions in this position in man.

The most recent accounts of the single nerve fibres in the substantia propria of the cornea are by Nageotte (1932) and Boeke (1935). Nageotte, however, is more concerned with the relationship of the Schwann cells to the axons than with their terminations. He apparently did not observe any nerve terminals ending freely, for he does not mention them; moreover, he believes that all axons in the substantia propria are related to Schwann cells, which we have shown not to be the case. He gives a good description of the close relation between axis cylinders and occasional corneal corpuscles, and also describes at some length the numerous beaded cytoplasmic processes associated with fixed and shrunken Schwann cells. Boeke describes the presence of 'isolated' axons in the substantia propria, but states that they are surrounded throughout their course by the cytoplasm of what he terms 'orthoklone' connective tissue cells. We are not able to agree with Boeke, for we have shown that, although single axons may be intimately related to the cytoplasm of corneal corpuscles for some distance, they do not enter them. In addition, we have shown that single axons pursue the greater part of their course in the naked state. We agree with Boeke in that we could not demonstrate epineurial sheaths surrounding nerve bundles, nor could we demonstrate endoneurial tubes in the cornea.

Dogiel (1891) described a number of special 'Nervenendapparaten' in the substantia propria of the cornea in man; they took the form of end-loops, hooks, coils and plates, and were situated in the periphery approximately $1\frac{1}{2}$ mm. from the limbus, mainly in a plane just beneath Bowman's membrane; such endings were even more numerous in the conjunctiva, where some of them took the form of Krause end-bulbs. Attias was unable to confirm the presence of any coils in the substantia propria, but occasionally saw nerves ending in the form of loops, hooks and plates about 1 mm. from the limbus. Boeke (1935) claimed to have demonstrated 'end coils' similar to those described by Dogiel, but when their respective drawings are compared the terminals do not seem to resemble each other at all closely.

We have been unable to confirm the findings of Dogiel, Attias or Boeke. In view of the observations in our previous paper (Weddell & Zander, 1950), we are convinced that the appearances which they describe are artefacts appearing in the course of the preparation of the specimens for histological examination. Although we have confirmed the existence of Krause end-bulbs in the conjunctiva in man, they are not so numerous as depicted in Dogiel's drawings, and we believe that some of the terminals he saw were also artefacts arising in a similar manner. We have not seen Krause end-bulbs in the conjunctiva of the rabbit, guinea-pig or rat.

In 1862, Kühne stated his belief that nerve fibres terminated within the cytoplasm of the corneal corpuscles. This announcement aroused much controversy and his views were not generally accepted; Waldeyer & Izquierdo (1880), Lipmann (1869) and Königstein (1875, 1877) agreed with Kühne, but Engelmann, Koelliker (1848, 1866), Petermoeller (1869), Hoyer, Ranvier, Dogiel, Virchow and Attias stated that they were not able to confirm his findings. Virchow, in his review in 1910, said that the evidence was overwhelmingly in favour of the view that nerves do not end in the cytoplasm of the cells of the substantia propria and that it should be accepted that they do not do so. However, Boeke & Heringa (1923) and Boeke (1935) restated the problem, for they claimed to have demonstrated nerve fibres running in the cytoplasm of the corneal corpuscles and terminating within them. Reiser (1936–7) also adhered to the views of Boeke. In contrast to these observations, Nageotte & Guyon (1926) and Martinez (1940) were convinced that there were no intracellular nerve fibres in the substantia propria. Martinez even denied the existence of free terminals, and believed that the nerve fibres in this situation ended in the form of an extracellular protoplasmically continuous network.

In view of our observations on fresh untreated corneas under phase-contrast conditions we believe that Virchow was correct, and that the findings of Boeke and Reiser are artefacts resulting from shrinkage during the course of preparation of the tissue for microscopical examination as well as non-specific impregnation methods. However, nerve fibres in the substantia propria occasionally end on the surface of the corneal corpuscles and also occasionally on the surface of histiocytes which are found in the cornea. If staining with methylene blue is prolonged, the histiocytes in particular take up the dye and their cytoplasm becomes filled with blue granules. Under such circumstances nerves may indeed appear to end intracellularly, and we believe that appearances similar to this would account for Boeke's observations and also for the observations of Dogiel and Egorow in relation to their special 'Nervenendapparaten'.

Nerve fibres in the corneal epithelium have variously been supposed to end in the following ways:

(1) As 'end-nets'—the nerve fibres forming a protoplasmically continuous network throughout the epithelium.

(2) In the form of end-nets and terminal knobs.

(3) Freely and in the form of terminal knobs.

(4) In the form of terminal knobs alone.

(5) In special 'Nervenendapparaten'.

(6) Within the cytoplasm of the epithelial cells.

(7) As free nerve terminals lying between the epithelial cells.

Observations under phase-contrast conditions indicate that the corneal epithelial nerves end in one of two ways: (1) very rarely, in the form of protoplasmically continuous finely beaded nerve nets, such nets being formed only by the fusion of daughter axons derived from neighbouring nerve fibres; (2) more commonly, in the form of beaded axons which lie in between the cells composing the epithelium, the terminal beads usually being larger than the preceding beads. The terminal beads, however, do not seem to us to be in any way specialized structures, as claimed by Attias, who, incidentally, denied the existence of protoplasmically continuous nets. Our observations are more in accordance with those of Virchow than of any other

author's, save that we have shown that 'end-nets' are very rare. We have not been able to demonstrate any organized or complicated nerve terminals in the corneas of the species we have examined.

So far, we have been unable to demonstrate any endoneurial tubes by the methods we have employed, nor indeed do there seem to be any clearly demonstrable connective sheaths surrounding any of the nerve bundles present. It seems that the bundles lie free between laminae of the connective tissue fibres which compose the substantia propria, being flattened in an antero-posterior plane. The axons appear to lie on the surface of a longitudinal series of Schwann cells when travelling in bundles, but when travelling singly in the substantia propria they lose their Schwann cell pathway shortly after leaving the nerve bundle and proceed naked for distances up to three-quarters of the diameter of the cornea lying among the connective tissue elements. This observation is in disagreement with Nageotte's (1932) statement that there are no naked nerve fibres in the substantia propria.

Following prolonged methylene-blue staining, after silver impregnation, and following the examination of fresh untreated tissues under phase-contrast conditions, we were unable to demonstrate Schwann cells in the corneal epithelium, the axons pursuing their course in the naked state. In this, we are in agreement with Nageotte (1932).

(2) *Corneal innervation and the neuron theory*

Before the appearance of Hoyer's and Cohnheim's (1866) papers it was generally accepted that corneal nerve fibres ended in the form of a net within the substantia propria. It was believed, as far as can be judged by the descriptions and terminology used, that all the nerves fused with one another in the form of a protoplasmically continuous network. The demonstration by Cohnheim (1866) of nerve fibres in the corneal epithelium indicated, however, that this system of 'end-nets' could no longer be regarded as the ultimate termination of the corneal nerve fibres.

Virchow's and Attias' descriptions of corneal innervation, still acknowledged as classical, show that they probably regarded the neuron theory as acceptable. Thus, for instance, they were at pains to point out that the terms 'net' and 'plexus' should be avoided. Nevertheless, Virchow (1910) retained the term 'Schlussnetz' for the plexiform arrangement situated just beneath Bowman's membrane.

Unfortunately, it is not possible to determine from the descriptions given by previous authors exactly what they believed the position of corneal innervation to be in relation to the neuron theory. This is due both to the multiplicity of terms used and to the difficulty of defining their meaning with any precision. For instance, the plexiform arrangement just beneath Bowman's membrane is referred to as a 'Schlussnetz' by Koelliker (1848), as an 'Endnetz' by His (1863), and as a 'Terminalnetz' by Saemisch (1862). Cohnheim in 1867, however, spoke of 'mehreren in verschiedenen Ebenen übereinander liegenden Netzen und Geflechten', to describe the pattern of innervation of the cornea; a definition which is not at variance with any of the observations we have made, yet is, we believe, not precise enough to be used as an argument for or against the validity of the neuron theory.

In 1873, Hoyer used the term 'mehrschichtiges Geflecht' to describe the general pattern of the nerves in the cornea and even gave a separate term, 'sub-basalar

plexus', to the plexiform arrangement just beneath Bowman's membrane. Ranvier (1881) speaks of a 'plexus fundamentale' to describe the plexiform arrangement just beneath Bowman's membrane, and Bach (1896) has accepted this description and expressed it in German as the 'Fundamentalplexus'. In 1887, Schwalbe describes this plexiform arrangement as a 'Grundplexus'. Dogiel (1890) described nerve fibres as occurring throughout the cornea, but specifically named the plexiform arrangement just beneath Bowman's membrane as a 'Hauptgeflecht'. Virchow (1910), realizing the unsatisfactory nature of the terms used by previous authors, introduced the word 'Gerüst' to describe the arrangement of the nerves in the larger bundles, a better term, but, as he himself admitted, still not entirely satisfactory, for it implies a rigidity which does not in fact exist. Nakajima's (1930) publication is very difficult to interpret, for he used a combination of both Attias's and Virchow's terms.

All the terms referred to are readily understandable in the light of our observations, but unfortunately they all avoid the issue as to whether or not the nerve fibres contained in the plexiform arrangement of nerve bundles enter either partially or completely into protoplasmic continuity with one another. Thus none of the publications before the time of Attias (1912) can be used as evidence for or against the validity of the neuron theory.

In 1935–6 Reiser, using silver-impregnation methods, deliberately used the anatomy of corneal innervation to attack the validity of the neuron theory, claiming that all corneal nerve fibres enter a protoplasmically continuous 'praeterminales Netzwerk', this preterminal network in turn giving rise to a 'terminal reticulum' which surrounds and penetrates each corneal cell like a honeycomb. His objections to the neuron theory were further strengthened by the following experimental observations. Reiser extirpated the Gasserian ganglion in rabbits and demonstrated that after 30–72 hr., although signs of nerve degeneration were apparent in nerve trunks, there was very little change in the 'preterminal network' and no change at all in the 'terminal reticulum'. He explained these findings on the grounds that undamaged nerve fibres entering the cornea from the conjunctiva ended in the protoplasmically continuous 'terminal reticulum' and were sufficient to keep the 'preterminal network' partly, and the 'terminal reticulum' wholly, in a normal condition throughout the cornea despite the removal of the majority of the corneal nerves.

Our observations do not lead us to the same conclusions as Reiser's. When methylene blue-staining of the cornea is prolonged beyond the time found necessary to stain only the axis cylinders specifically, the Schwann cells also take up the dye and their cytoplasm appears to contain a network of fine fibrils (Weddell & Zander, 1950). Under such conditions it is difficult to exclude the possibility that axis cylinders do not give rise to the stained threads seen in association with the Schwann cytoplasm. Moreover, these stained threads appear to fuse with similar processes from neighbouring cells, particularly at the level of the plexiformly arranged nerve fibres. The pattern given is, in fact, very similar to that described by Reiser as the 'preterminal network'. In our opinion such an appearance is the result of non-specific staining and cannot be used as an argument for or against the neuron theory. That it is the Schwann cytoplasm which becomes stained after prolonged treatment with methylene blue, and that the fibrils seen are not in direct continuity with the nerve axis cylinder, can be demonstrated in two ways. If nerves supplying the cornea

are sectioned and a sufficient time left for all the axis cylinders to degenerate—a period of between 20 and 25 days—it is still possible to stain the Schwann cells with methylene blue and to obtain a picture which is in every way similar to that seen after prolonged staining with methylene blue before nerve section, save that the axis cylinders are either completely absent or else represented by small blue-stained fragments which remain in the position of the original course of the axons (Pl. 4, fig. 32). Secondly, if fresh untreated corneal tissue is examined under phase-contrast conditions, the axons are seen to course along the surface of the Schwann cell cytoplasm and to be free from processes which enter the cells. Moreover, under these conditions the Schwann cells are seen to be unshrunken and devoid of beaded processes if the tissue is examined immediately after removal from the animal. In preparations which have been mounted in saline and ringed with vaseline for some 60 min. the Schwann cells are shrunken; their cytoplasm appears granular and commencing to resolve itself into beaded processes. These are even better developed sometime later.

In Reiser's demonstration of the persistence of the 'terminal reticulum' after nerve section, periods of not longer than 72 hr. were allowed for nerve degeneration. Experimental work now in progress has shown that this period is too short, in the case of the cornea, for any but the earliest signs of degeneration to become manifest, and it is therefore not surprising that fine axons persisted in his preparations 72 hr. after nerve section, a fact which, together with the use of a non-specific histological method, would naturally have led him to the conclusion that he drew.

We, like Boeke (1935), have been unable to demonstrate by any method at our disposal the honeycomb-like network of fine fibrils of Reiser's 'terminal reticulum'. Reiser himself published neither photographs nor drawings illustrating these elements in relation to the cells of the corneal epithelium which in any way correspond with his descriptions. Consequently, it is not possible for us to make any further observations on this point.

We have never seen fusion between daughter axons originating from neighbouring nerve fibres. On the other hand, in the epithelium, in methylene blue-stained preparations, we have occasionally seen protoplasmically continuous nets formed by the fusion of daughter axons originating from the same parent fibre. It was probably observations similar to these which led Martinez, who used silver techniques, to have serious doubts about the absolute validity of the neuron theory. These doubts can, we believe, be resolved only by the examination of nerve fibres in whole preparations, and thus it would not have been possible for Martinez, when examining silver-impregnated sections, to have been more definite than he was. For the reasons just discussed, therefore, we do not believe that there is any evidence that corneal innervation can be used in any way to attack the validity of the neuron theory.

(3) *Corneal innervation and the autonomic nervous system*

Boeke (1935) claims that in the larger bundles in the substantia propria of the cornea there are two types of nerve fibre: somatic fibres and what he terms a 'sympathetic ground plexus'; but he does not indicate why he believes that the 'ground plexus' is of autonomic origin.

We have been able to produce photographs from specimens which are comparable

in every way with the pictures published by Boeke. However, we again had to employ the methods of non-specific staining or impregnation which we used to demonstrate the 'preterminal network' of Reiser. This fact, together with the results of further degeneration experiments, have convinced us that the 'sympathetic ground plexus' in corneal nerve bundles also consists of stained cytoplasmic threads of shrunken and distorted Schwann cells. In preliminary observations following a series of cervical sympathetic neurectomies in the rabbit, we were unable to see any diminution in the number of nerve fibres present in the cornea, or any nerve fibres undergoing degenerative changes. Moreover, a 'sympathetic ground plexus' could still be demonstrated by the use of non-specific histological methods. These experiments are not, of course, conclusive for there are many autonomic ganglion cells scattered along the course of the cerebral vessels, some of which might supply the cornea, but they do suggest that the cornea does not receive a large sympathetic nerve supply. In this we are in agreement with Boeke (1935). We disagree only as to the existence of the 'sympathetic ground plexus' in the larger nerve bundles: a system which Boeke believes to persist, despite the removal of all the nerve fibres supplying the tissues in which they are found.

In a recent paper (Rodger, 1950) reference is made to the fact that the cornea contains sympathetic nerve fibres which are distinguishable from somatic nerve fibres on morphological grounds. The evidence upon which this statement is based is not given. Moreover, the methods used to demonstrate nerve fibres are, in our opinion, quite inadequate for the solution of this problem.

It seems possible that on rare occasions there are a few autonomic nerve fibres to be found in the cornea, for we have seen cells which resemble autonomic ganglion cells (Pl. 3, fig. 22) in three out of over 300 corneas which we have examined. Cells of this type have not been described previously; in fact the presence of ganglion cells has been denied by Ernyei (1934) and Rodger (1950); this is hardly surprising, for if the cells we have described are ganglion cells, they must be very rare. The existence of stray or aberrant autonomic ganglion cells in the distribution of the 5th cranial nerve is common, however, and perhaps their occasional presence in the cornea need cause no surprise (Boyd, 1950).

(4) *Correlation of anatomical with physiological observations*

It has now been demonstrated on a number of occasions that a sensation akin to touch can be aroused from the cornea by a light stimulus after the operation of trigeminal tractotomy, which has the effect, in successful cases, of abolishing all corneal pain sensibility. This observation can most easily be explained on the basis of the presence of tactile as well as pain nerves and terminals in the cornea.

When a number one nylon suture is lightly applied to, or drawn across, the corneal epithelium, it arouses a sensation of pain. Two minutes following the instillation of a dilute solution of Pantocaine into the conjunctival sac, however, pain can no longer be aroused in this way, but a sensation akin to touch is perceived whenever the stimulus is applied so as to deform the cornea. The sensation of 'touch' can in turn be abolished by the instillation of a higher concentration of the same anaesthetic. Observations of a similar nature were made by Goldscheider & Brückner in 1919.

In the light of our anatomical findings it is tempting to suggest that these observations can be explained in the following way. The nerves ending in the corneal

epithelium in the form of free-beaded terminals subserve pain; those ending in the substantia propria, also in the form of free-beaded terminals, subserve tactile sensibility.

If this explanation is true, it is of distinct interest and importance, for it suggests that the form in which sensory nerves terminate peripherally cannot necessarily be used as an argument in favour of the type of sensation which will be perceived if they are stimulated.

Tower has shown that a single ciliary nerve fibre transmits action potentials when stimuli are applied over a wide area of the cornea and, in addition, that these sensory units are physiologically independent and overlap one another. The anatomical basis for these physiological demonstrations is now clear.

The arrangement of the nerve terminals in the epithelium in 'depth' rather than as a series of interweaving fibres in a single plane, as in the skin, suggests, on the basis of the observations of Weddell *et al.* (1948), that the quality of pain sensibility aroused from the cornea will be similar to that which tends to give rise to autonomic responses when evoked from the skin.

The significance of the plexiform arrangement of the nerve fibres in the cornea is still unexplained unless it is assumed that the axon terminations have other functions to perform in addition to their sensory role. Such a distribution might be expected if, for instance, the axon terminations behave in a manner in which Lewis (1942) suggested that the 'nocifensor' nerves behave in the skin, but such properties have yet to be demonstrated.

SUMMARY

1. A study of the innervation of the cornea in a selected series of vertebrates is presented.

2. A variety of histological methods was used to determine: (*a*) the general arrangement of the nerve fibres, (*b*) the structural details of these fibres and their terminations, and (*c*) their relationship to the tissues which surround them.

3. The observations are discussed in relation to: (*a*) the literature, (*b*) the neuron theory, (*c*) their functional significance.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. From a methylene blue-stained (intravenous drip method) rabbit's cornea. It shows the arrangement of the subconjunctival and episcleral nerve bundles. (×80.)

Fig. 2. An infra-red flash photograph from a methylene blue-stained rabbit's cornea *in situ* in the living anaesthetized animal. It shows small nerve bundles arranged in a characteristic manner lying among blood vessels at the corneo-scleral junction. The arrows point to the nerve bundles. (×120.)

Fig. 3. From a silver impregnated monkey's cornea. It shows in more detail the arrangements seen in fig. 2. (×350.)

Fig. 4. From a methylene blue-stained rabbit's cornea (intravenous drip method). It shows a bundle at the corneo-scleral junction dividing into three branches. Simple pictures of this sort are not common. (×150.)

Fig. 5. From a methylene blue-stained rabbit's cornea. It shows axon multiplication at the point of division of a small nerve bundle at the corneal limbus. Such pictures are rare. (×200.)

Fig. 6. From a methylene blue-stained rabbit's cornea. It shows a nerve bundle at the corneo-scleral junction which contains a single myelinated nerve fibre. (×400.)

Fig. 7. From the periphery of a methylene blue-stained rabbit's cornea, and shows a myelinated axon in a small nerve bundle entering the cornea giving rise to an unmyelinated daughter axon. (×280.)

Fig. 8. From the periphery of a methylene blue-stained monkey's cornea and shows a number of myelinated axons. One of the myelinated axons is giving rise to two daughter myelinated axons. This is the only example of such an occurrence we have seen. (×290.)

Fig. 9. From a methylene blue-stained rat's cornea. It shows an axon taking a recurrent course in a small nerve bundle in the substantia propria. (×280.)

Fig. 10. From a methylene blue-stained rabbit's cornea and shows a daughter axon leaving a small nerve bundle in the substantia propria and subdividing again almost at once. This kind of picture is rare. (×400.)

Fig. 11. From a silver impregnated rabbit's cornea. It shows an axon dividing in the substantia propria into two branches each of a different diameter. (×550.)

Fig. 12. From a methylene blue-stained rabbit's cornea and shows axon multiplication in a small nerve bundle in the substantia propria. Note the abrupt change in direction of one of the daughter axons, and the slight swelling at the point of division. (×650.)

Fig. 13. From a methylene blue-stained rabbit's cornea following alcohol fixation. It shows axon multiplication at the point of division of a small nerve bundle in the substantia propria. Note the exaggerated swelling of the axis cylinder in this instance. Such swellings are difficult to distinguish from 'ganglion cells' or Schwann cell nuclei. (×650.)

Fig. 14. From a methylene blue-stained (slightly overstained) rabbit's cornea. It shows a branch leaving a nerve bundle in the substantia propria. The axons in the branch are flattened in a plane at right angles to that in which the axons of the main nerve bundle are flattened. (×390.)

Fig. 15. From a methylene blue-stained rabbit's cornea. Bundles of axons are seen to be passing in different directions and again to be flattened in different planes as they pass between the layers of the substantia propria. (×420.)

Plate 2

Fig. 16. From a methylene blue-stained guinea-pig's cornea. It shows a nerve bundle terminating in the plexiform arrangement of nerve fibres. (×300.)

Fig. 17. From a methylene blue-stained rabbit's cornea. It shows the plexiformly arranged nerve fibres lying deeply (out of focus) in the centre of the cornea and giving rise to beaded fibres (in focus) in the epithelium. (×150.)

Fig. 18. From a gold impregnated rabbit's cornea. It shows a nerve bundle halfway between the sclera and the centre of the cornea dividing into a number of branches. (×250.)

Fig. 19. From a methylene blue-stained guinea-pig's cornea. It is somewhat 'overstained', but gives a good idea of the way nerve bundles divide after entering the cornea. Almost all the cornea is seen in the picture. (×90.)

Fig. 20. From a methylene blue-stained rabbit's cornea. The tip of the incision is approximately at the centre of the cornea. A large nerve bundle can be seen passing from one limbus to the other equatorially across the cornea, and another bundle can be seen joining it near the tip of the incision. (×60.)

Fig. 21. From a gold impregnated rabbit's cornea. It shows a medium-sized nerve bundle passing from one limbus to the other across the centre of the cornea. Almost the whole cornea is represented. (×90.)

PLATE 3

Fig. 22. From a methylene blue-stained rabbit's cornea. It shows a nucleated 'ganglion' cell in the substantia propria. Such cells are very rare. (×700.)

Fig. 23. From a methylene blue-stained rabbit's cornea. It shows an axon which gives rise to terminals in the substantia propria arising from a nerve bundle. (×350.)

Fig. 24. From a methylene blue-stained guinea-pig's cornea. It shows an axon which gives rise to terminals in the substantia propria arising from a nerve bundle. This figure should be compared with figs. 23 and 25. (×300.)

Fig. 25. From a methylene blue-stained rabbit's cornea. It shows an axon which gives rise to terminals in the substantia propria arising from the plexiform arrangement of nerve fibres. (×200.)

Fig. 26. Also from a methylene blue-stained rabbit's cornea, showing the complex pattern formed by nerve fibres before they terminate in the substantia propria. (×350.)

Fig. 27. From a methylene blue-stained rabbit's cornea. It shows a complete spray of terminal beads in the substantia propria. (×350.)

Fig. 28. From a methylene blue-stained rat's cornea. It also shows the complex pattern formed by a nerve fibre terminating in the substantia propria. This figure should be compared with fig. 26. (×350.)

Fig. 29. From a silver impregnated rabbit's cornea and shows an axon (associated with a Schwann cell nucleus and cytoplasm) in the substantia propria passing in close relation to a corneal corpuscle and giving rise to what appears to be a branch which ends on its surface. (×800.)

Fig. 30. From a methylene blue-stained rabbit's cornea. It shows a sheet of corneal epithelium lining the anterior chamber which has been stripped away from Descemet's membrane. No nerve fibres are seen but there are a few circular blobs of stain lying between the cells. (×400.)

PLATE 4

Fig. 31. From a rabbit's cornea which has been deliberately overstained with methylene blue. It shows a nerve bundle in which the nerve axis cylinders have been stained as well as the Schwann cells. The Schwann cell nuclei can be identified easily, but the cytoplasm is so shrunken and distorted that it is impossible to distinguish some of the cellular processes from nerve axis cylinders. (×45.)

Fig. 32. From the centre of a methylene blue-stained rabbit's cornea. The central two-thirds of this cornea had been excised and replaced as an autoplastic graft 20 days previously. It shows Schwann cell nuclei surrounded by shrunken and distorted cytoplasm. No nerve fibres can be seen. Compare this figure with figure 31. (×450.)

Fig. 33. From a silver impregnated rabbit's cornea. It shows a single nerve fibre related in the lower half of the picture to a Schwann cell; when traced distally the axon proceeds in an apparently naked state. (×700.)

Fig. 34. From a monkey's cornea. The preparation is a fresh unstained razor slice. It shows the appearance of a small nerve bundle in the substantia propria under phase-contrast conditions. Note the Schwann cell nuclei and cytoplasmic pathways. (×450.)

Fig. 35. From a gold impregnated rabbit's cornea. It shows two axons in the substantia propria. The thick axon is related to Schwann cytoplasm whilst the thin axon is apparently naked. (×480.)

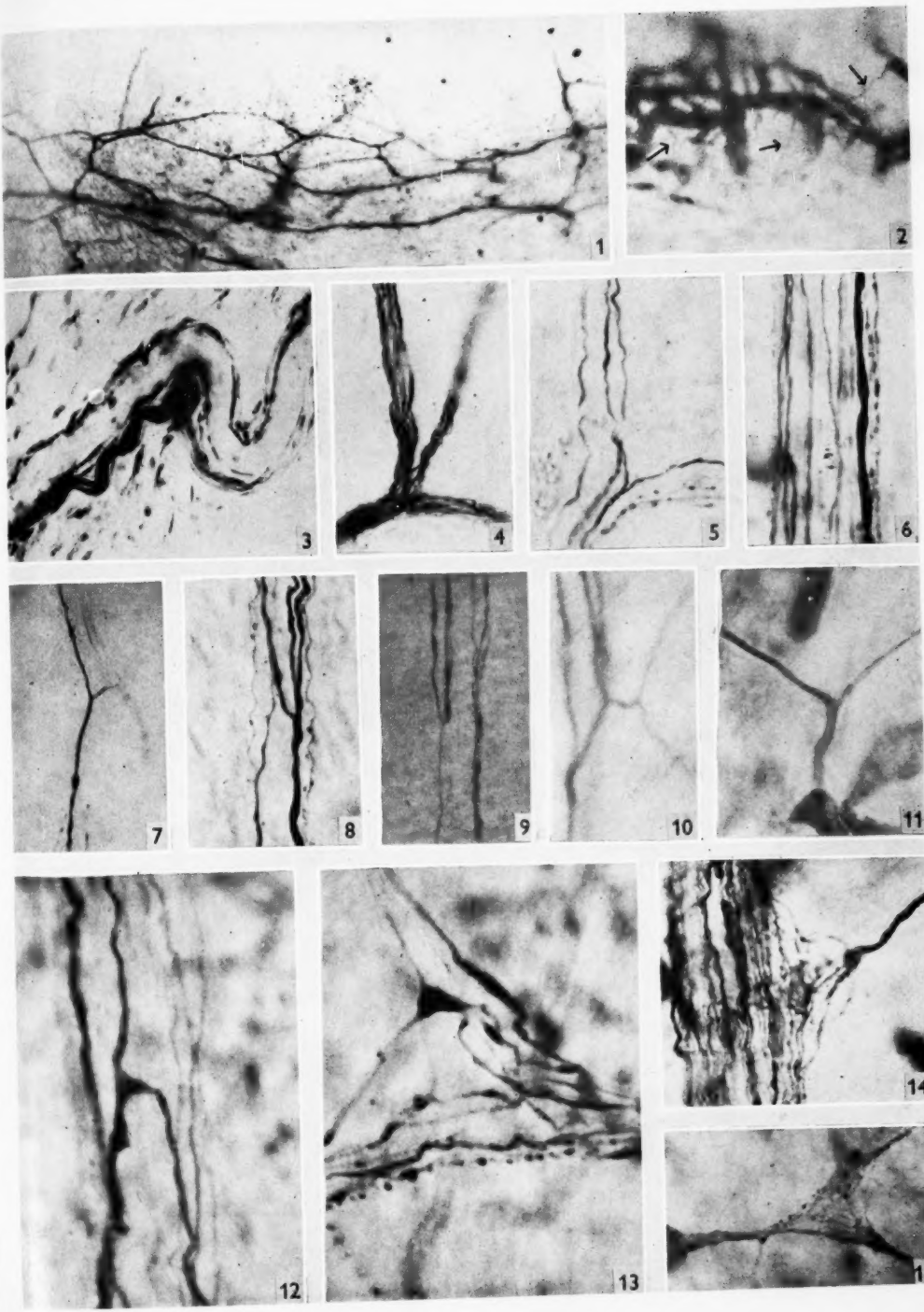
Fig. 36. From a methylene blue-stained rabbit's cornea. It shows the peculiar course taken by a number of single axons in the substantia propria. Compare with fig. 37. (×600.)

Fig. 37. From a rabbit's cornea examined under phase-contrast conditions. It shows a single axon in the substantia propria behaving in a manner resembling that seen in fig. 36. (×600.)

Fig. 38. From a silver impregnated rabbit's cornea showing a single nerve fibre in the substantia propria apparently terminating on the cytoplasm of a corneal corpuscle. Note the slight irregularity and splitting of the axon. (×900.)

Fig. 39. From a gold impregnated rabbit's cornea. It shows nerve bundles giving rise to a penicillium of beaded axons which ramify between the cells of the basal layer of the epithelium. (×350.)

Figs. 40, 41. Both are from gold impregnated rhesus monkey corneas. They are taken at different depths from the surface and illustrate clearly that there are no nerve fibres ramifying in the plane between Bowman's membrane and the basal epithelial layer. (×300; ×350.)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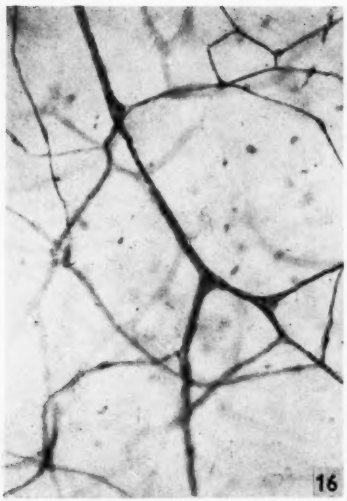
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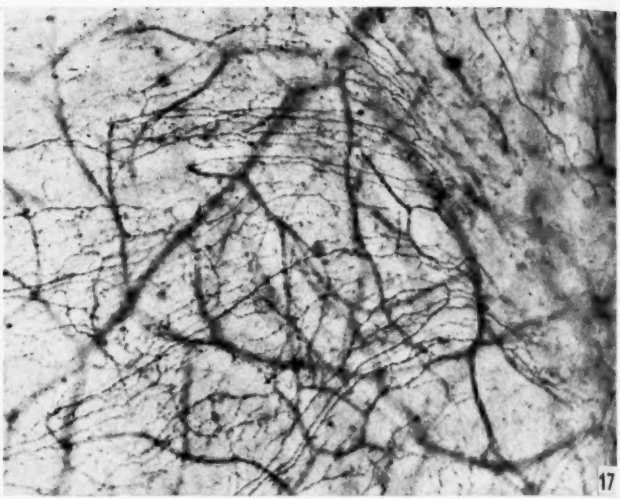
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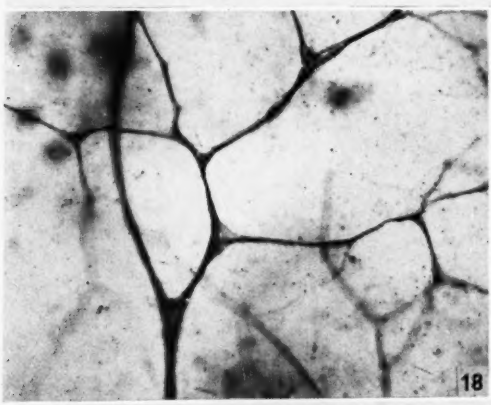
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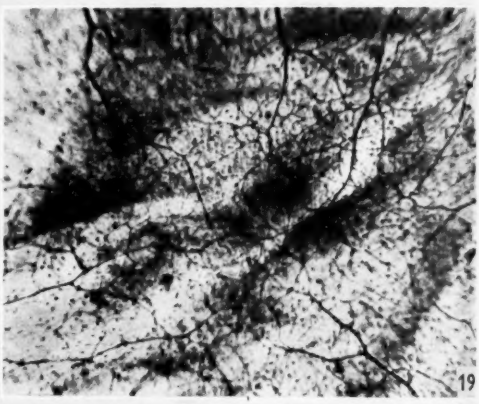
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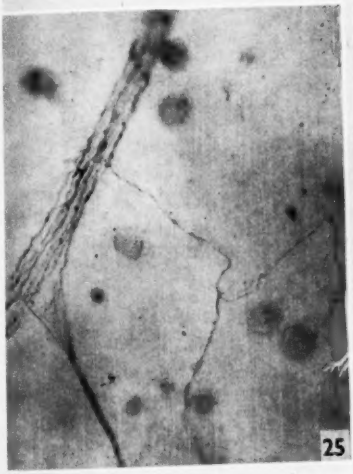
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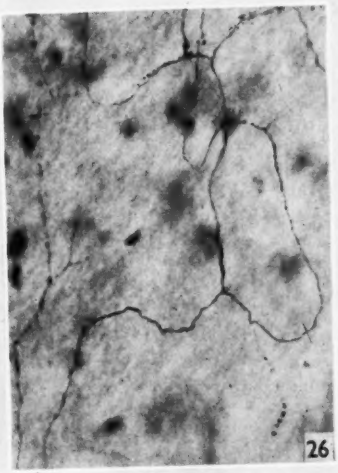
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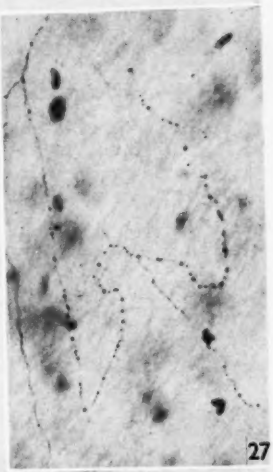
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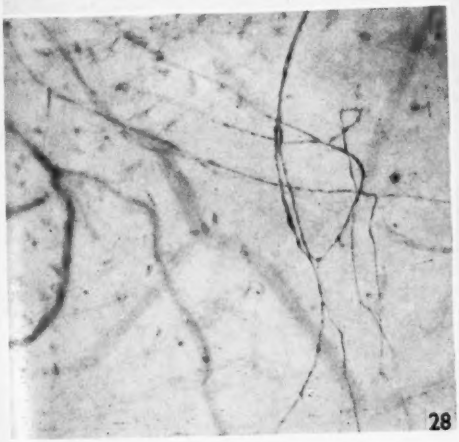
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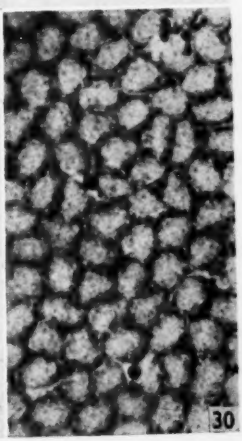
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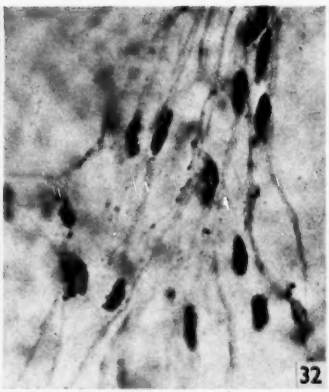
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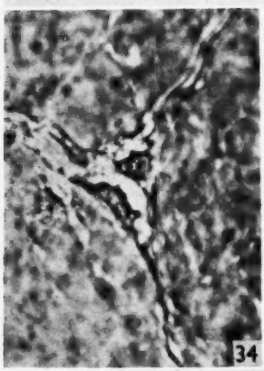
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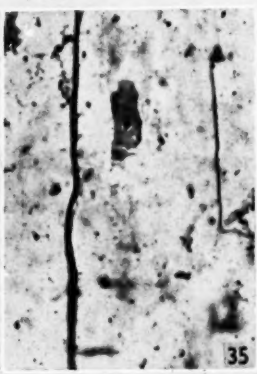
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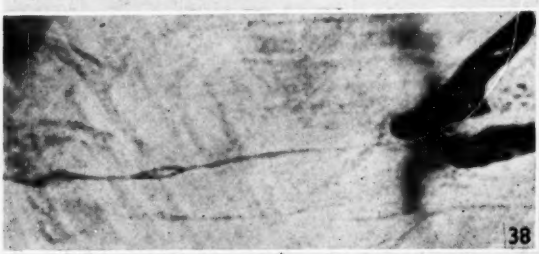
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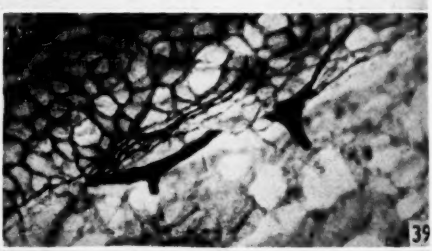
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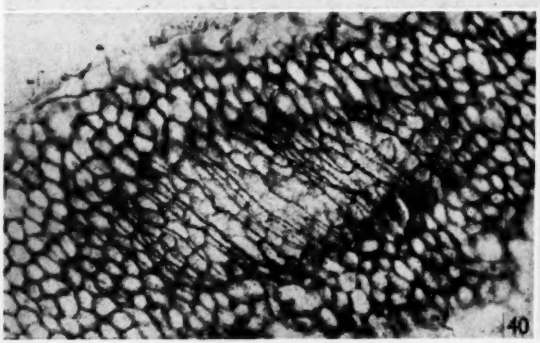
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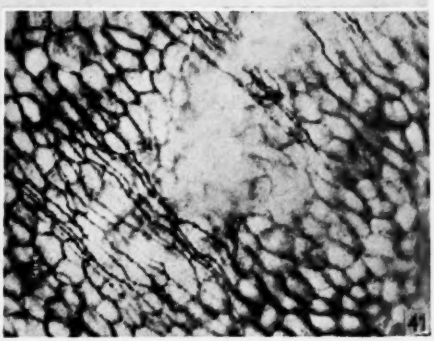
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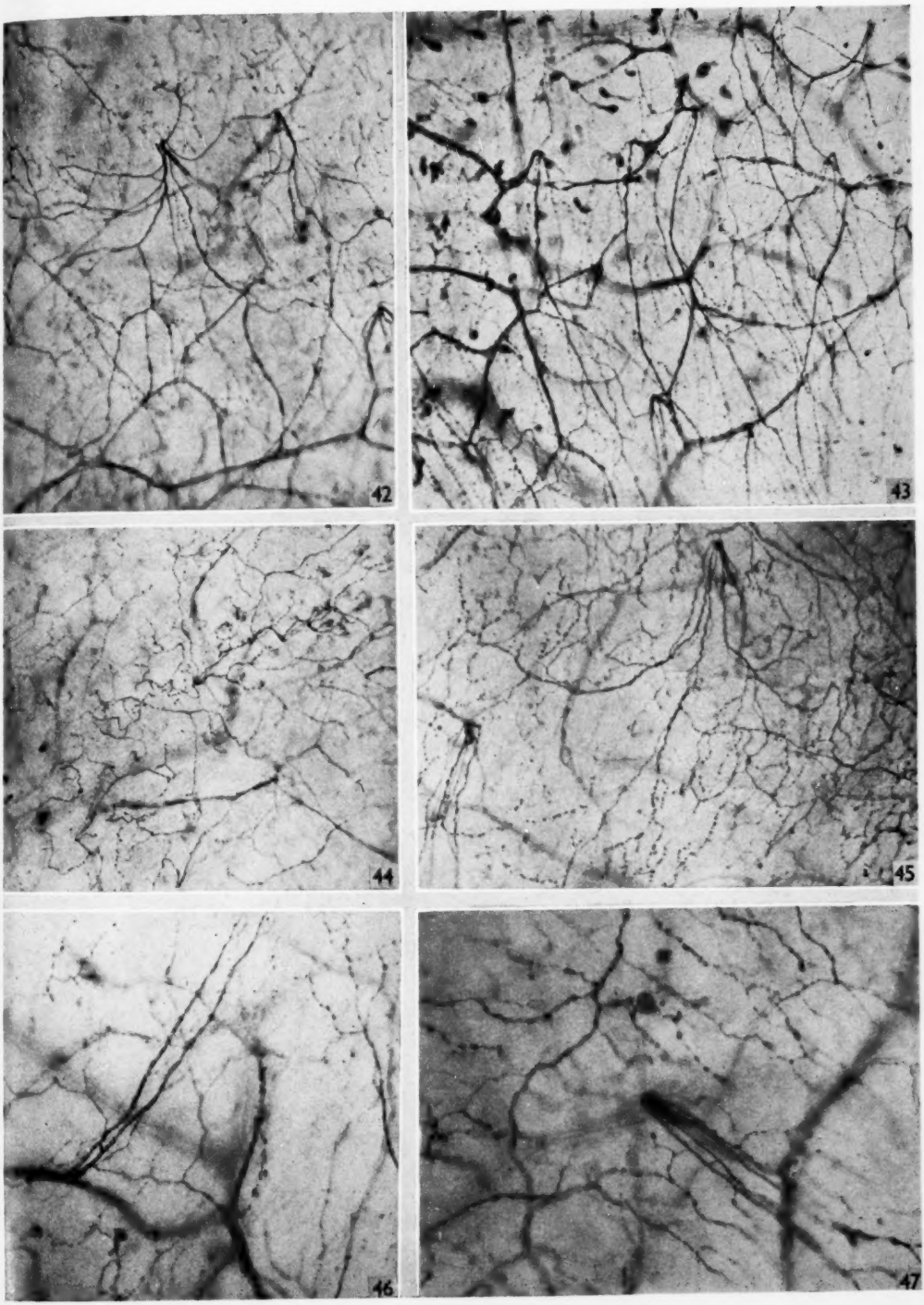
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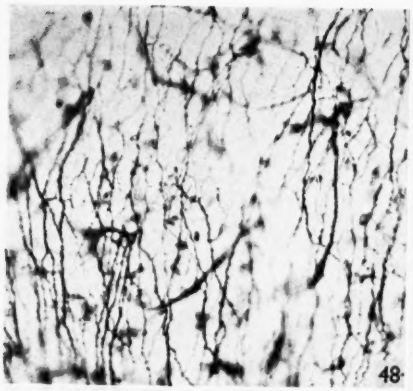
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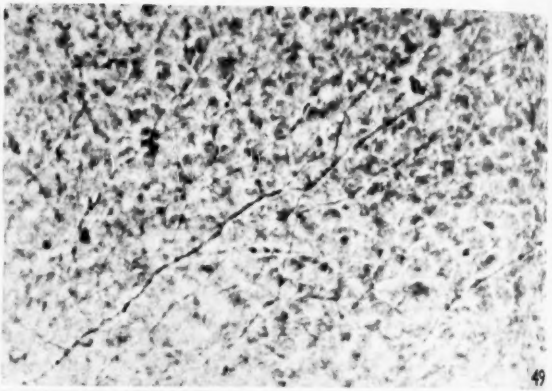
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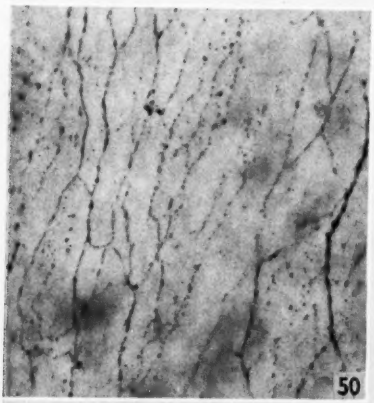
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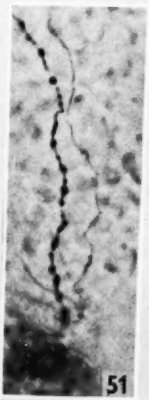
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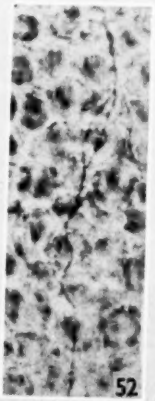
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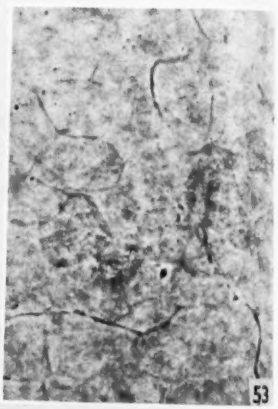
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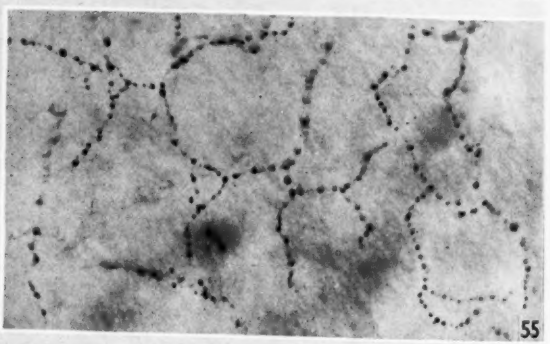
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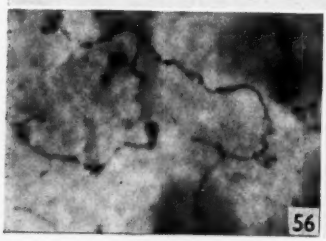
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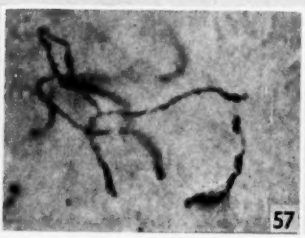
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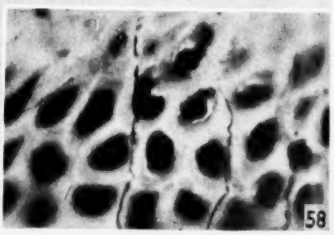
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PLATE 5

All figures are from methylene blue-stained preparations.

Fig. 42. From the periphery of a rabbit's cornea. It shows axons piercing Bowman's membrane, multiplying and passing in all directions in the epithelium. (×150.)

Fig. 43. From a position not far from the centre of a rabbit's cornea. It also shows axons piercing Bowman's membrane almost vertically, multiplying and passing in a radial direction in the epithelium. (×160.)

Fig. 44. From the periphery of a guinea-pig's cornea. It shows axons piercing Bowman's membrane, multiplying and passing in all directions in the epithelium. (×140.)

Fig. 45. From a position near the centre of a rabbit's cornea. It also shows axons piercing Bowman's membrane almost vertically, multiplying and passing in all directions in the epithelium. (×130.)

Fig. 46. From a rabbit's cornea and shows an axon dividing into two after piercing Bowman's membrane. (×280.)

Fig. 47. From a rabbit's cornea and shows an axon dividing into numerous branches after piercing Bowman's membrane. (×300.)

PLATE 6

Fig. 48. From a methylene blue-stained rabbit's cornea. It shows axons ramifying at different depths in the epithelium. (×300.)

Fig. 49. From a monkey's cornea examined under phase-contrast conditions. It shows much the same thing as fig. 50, but the epithelial cell bodies can also be seen. (×350.)

Fig. 50. From a methylene blue-stained monkey's cornea. It shows beaded axons ramifying in the basal part of the epithelium. (×300.)

Fig. 51. From a tangential frozen section of a methylene blue-stained rabbit's cornea. It shows two beaded axons of different diameters situated in about the middle third of the epithelium. The finer of the two axons shows very little beading. (×450.)

Fig. 52. From a rabbit's cornea. The preparation was a fresh untreated razor slice of corneal epithelium. The appearances are those seen under phase-contrast conditions. Note that the beads are very fine. (×600.)

Fig. 53. From a monkey's cornea. The preparation was a fresh untreated razor slice of corneal epithelium examined under phase-contrast conditions *immediately* after removal. Note the narrow diameter of the nerve fibres, and the extreme fineness of the beads along their course. The nerve fibres and their terminals are all situated extracellularly. (×600.)

Fig. 54. From a gold impregnated rabbit's cornea. It shows nerve fibres in the epithelium. The fibres towards the surface are beaded. (×500.)

Fig. 55. From a methylene blue-stained monkey's cornea. It consists solely of epithelium, which has been stripped off the substantia propria. It shows beaded nerve fibres terminating extracellularly in end-beads passing in between cells in the middle-third of the epithelium. (×500.)

Fig. 56. From a methylene blue-stained rabbit's cornea. It is from a tangential frozen section and shows finely beaded nerve fibres lying between the corneal epithelial cells. (×500.)

Fig. 57. From a methylene blue-stained rabbit's cornea. It is also from a tangential frozen section and shows finely beaded nerve fibres in the epithelium. Note the enlarged end-beads. (×600.)

Fig. 58. From a silver impregnated vertical section of a dogfish's cornea. It shows fine nerve fibres becoming beaded as they approach the more superficial layers of the epithelium. They lie between the epithelial cells. The finest fibres cannot be demonstrated by this method. (×600.)

ANATOMICAL NOTE

A RARE ANOMALY OF M. FLEXOR DIGITORUM SUBLIMIS

By DHIRENDRANATH S. CHOWDHARY, *Department of Anatomy, S.N. Medical College, Agra, India*

The anomaly reported here was found in the course of dissection of a female body of about 35 years of age, and it was bilateral. The flexor digitorum sublimis had its normal origin, giving rise to two superficial bellies, a radial and an ulnar, and one deep belly. The superficial radial belly was continued as a tendon to the middle finger as usual, and the superficial ulnar belly was continued to the ring finger but its tendon passed superficial instead of deep to the flexor retinaculum (Figs. 1, 2).

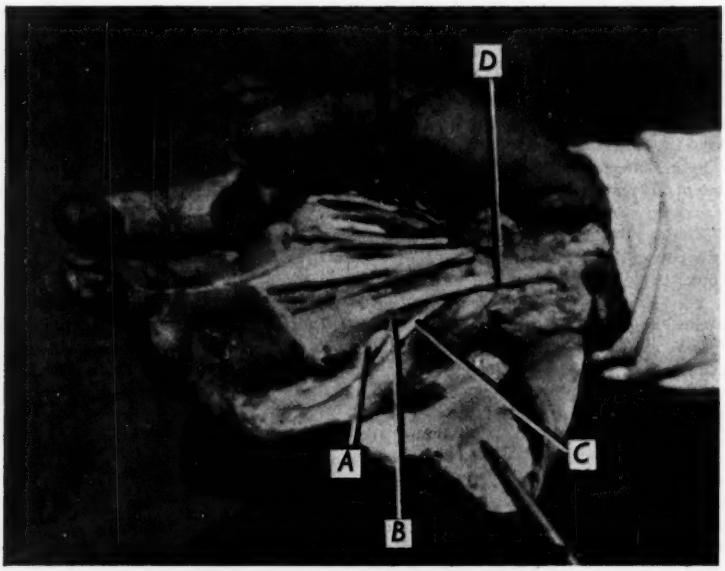


Fig. 1. Photograph of specimen to show the condition described. *A*, anomalous tendon in the palm; *B*, 4th lumbrical; *C*, tendon of flexor digitorum profundus; *D*, tendon of flexor digitorum sublimis passing superficial to flexor retinaculum.

The deep belly instead of dividing as usual into two bellies (cf. Wood Jones, 1944, fig. 96) continued as a single tendon to the index finger.

There was no sign in the forearm or wrist of any muscle belly or tendon destined for the little finger.

On opening the fibrous flexor sheath of the little finger there was found opposite the proximal phalanx a thin, flat, tendinous slip lying slightly superficial to the tendon of flexor profundus and between this on the medial side and the 4th lumbrical on the lateral side (Figs. 1, 2).

Proximally the slip arose in front of the 4th metacarpal as a condensation of the anterior interosseous fascia, and distally it was inserted like a normal sublimis tendon.

Wood Jones (1944, pp. 229, 230) states that the deep portion of the muscle is liable to considerable minor variations, and that it appears to be mainly devoted to the index finger. Le Double (1897, p. 97) mentions absence of a tendon to the little finger and its replacement by a slip arising from the flexor retinaculum or the palmar aponeurosis. But I have been unable to find any account of such a condition as I have described in the tendon to the little finger, nor of the passage of the tendon to the ring finger over the flexor retinaculum.

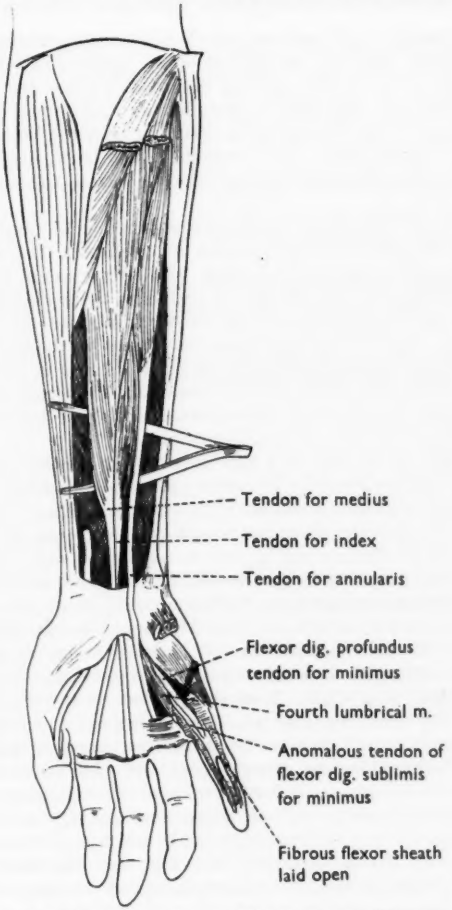

Tendon for medius
Tendon for index
Tendon for annularis
Flexor dig. profundus tendon for minimus
Fourth lumbrical m.
Anomalous tendon of flexor dig. sublimis for minimus
Fibrous flexor sheath laid open

Fig. 2. Drawing to show the condition in hand and forearm.

I wish to thank Prof. C. B. Singh for permission to report this anomaly, and Dr Miss U. Mehta for the drawing and Shri P. Upadhyaya for the photograph. I am very grateful also to Prof. G. Tondury, of Zurich, for the loan of a copy of Le Double's work.

REFERENCES

Le Double, A. F. (1897). *Traité des Variations du Système Musculaire de l'Homme*, **2**. Paris: Schleicher.

Wood Jones, F. (1944). *The Principles of Anatomy as seen in the Hand*, 2nd ed., pp. 229, 230. London: Baillière, Tindall and Cox.

REVIEWS

The Mammalian Adrenal Gland. By GEOFFREY H. BOURNE. (Pp. 1–239, Plates 1–15. 30*s*.) Oxford: Clarendon Press. 1949.

A number of monographs on the adrenal glands have been published in recent years, but they have been written from the standpoint of the pathologist, the physiologist, or the biochemist. In view of the rapidly growing importance which the suprarenal glands and their steroid hormones are beginning to assume, the appearance of a monograph by an anatomist, presenting our knowledge of the gland against the broad background of comparative anatomy, is particularly to be welcomed. The book, needless to say, is written with the customary scholarship and erudition we have learned to associate with Dr Bourne's writings.

The book commences with a valuable historical introduction, and a brief general survey of our knowledge of the adrenals in most classes of vertebrates. It is perhaps to be regretted that in this survey no reference has been made to the structure of these glands in birds, in which the intimate association of cortical and medullary tissue may be so striking a feature. This is all the more noteworthy since, as the author points out, the complete separation of medulla from cortex, and the surrounding of the former by the latter, seem to constitute as typical a mammalian characteristic as the presence of hair and nails. In view of the close relationship between the adrenals and body temperature, it is noteworthy that in the only two vertebrate groups which have become isothermic there has been a convergence of cortical and medullary tissue, on very different lines.

Dr Bourne's studies on ascorbic acid have given him a worthy place among the pioneer workers in the field of histochemistry. We must confess to a slight feeling of disappointment that, eminently qualified as he is to do justice to the topic, he has not in the present survey dealt at greater length with the more recent histochemical work on the suprarenal cortex. It is to be hoped that Dr Bourne will be able to rectify this omission in future editions.

The title of the book, *The Mammalian Adrenal Gland*, serves to draw attention to a somewhat irritating problem of terminology. In this country, we usually describe the glands as suprarenal, whereas in the American literature they are generally referred to as the adrenal glands. Though we follow our transatlantic colleagues in describing the medullary secretion as adrenalin, we nevertheless, with our customary conservatism, have retained the term suprarenal for the gland as a whole. More recently, we seem to have accepted the adrenotropic hormone of the pituitary, and have not attempted to substitute for it the more cumbersome suprarenotropic hormone. What, in fact, has been happening has been our gradual adoption of adrenal instead of suprarenal, and while one would hesitate to recommend to anatomists in this country a further revision of terminology, one wonders whether some compromise is not possible, such as officially recognizing the alternative name.

Dr Bourne has collected, and systematically presented, a mass of information not available from any other source. His monograph is bound to stimulate further interest in a gland whose hormones give fair promise of revolutionizing our concepts of bodily processes and tissue reactions.

J. M. YOFFEY

Le Système Neurovasculaire. By GUY LAZORTHES. (Pp. 300, 71 figs. 950 fr.) Paris: Masson et Cie. 1949.

This monograph on the anatomy, physiology, pathology and surgery of the neurovascular system is, as Prof. R. Leriche states in the preface, the best that has yet appeared on the subject. The author, Prof. Guy Lazorthes, is both an anatomist and neurosurgeon, and he has devoted ten years to a specialized study of the vascular nerves in every region of the

body. Our ignorance about many points concerning these nerves is familiar to every anatomist, and Lazorthes' work fills a real gap in our knowledge.

The monograph provides a well-balanced review of many previous publications and a record of extensive personal investigations. It is fully documented, although minor inaccuracies occur in certain of the references given. The arrangement is logical, so that information on any desired point is obtainable without much difficulty, but in subsequent editions it would be desirable to supplement the table of contents with subject and author indices.

The discussion of the vasomotor centres and pathways in the central nervous system and of their connexions with the sympathetic gangliated trunks and cranial and spinal nerves follows generally accepted lines, as does the description of the physiology. Lazorthes' main anatomical contribution lies in his descriptions of the nerves supplying most of the arteries and many of the veins; these are mainly founded on personal observations, but some are based on investigations reported by others. In a brief review it is impossible even to summarize the findings, and while they do not always coincide with those of other workers who have also devoted much time to the dissection of these delicate nerves, undoubtedly no anatomist interested in the autonomic nervous system can afford to overlook this monograph.

One does not feel competent to evaluate the pathological and surgical sections, but as they merit the unqualified approval of a great authority such as Leriche, it is fair to assume that they are excellent.

Like many other French books, the binding and format fail to do justice to the contents, and it is irritating and surely unnecessary to have to cut almost every page before it can be read.

G. A. G. MITCHELL

Radiographic Atlas of Skeletal Development of the Hand and Wrist. By WILLIAM WALTER GREULICH, M.A. Ph.D. and S. IDELL PYLE, M.S. (Pp. ix+190, 80*s*.) Stanford University Press. 1950.

The book is subdivided into four main sections—The Rationale and Technique of Assessing the Developmental Status of Children from Roentgenograms of the Hand and Wrist; Male Standard, Female Standard and Maturity Indicators of Individual Bones and Epiphyses.

The authors show conclusively that in the normal child development proceeds harmoniously for all parts. They believe that in the skeleton, especially that of the hand and wrist, they have a concise guide to the changes associated with progressive growth and maturity. The authors found that there was a close relationship between the maturity changes in the skeleton and the reproductive system which in turn is responsible for bodily maturity. The material on which the work is based is so extensive that for each of the standards in the atlas one hundred films of children of the same sex and age were available. Valuable information is also presented on the effects of precocious puberty, hypogenital states, and illness on the development of wrist and hand.

This book should be consulted by all who are interested in the growth of the child.

The authors are to be congratulated on the fine reproduction of the skiagraphs, which with few exceptions (figures on pages 83, 89 and 131) are uniformly good. The publishers are also to be complimented on the excellence of the format.

W. J. HAMILTON

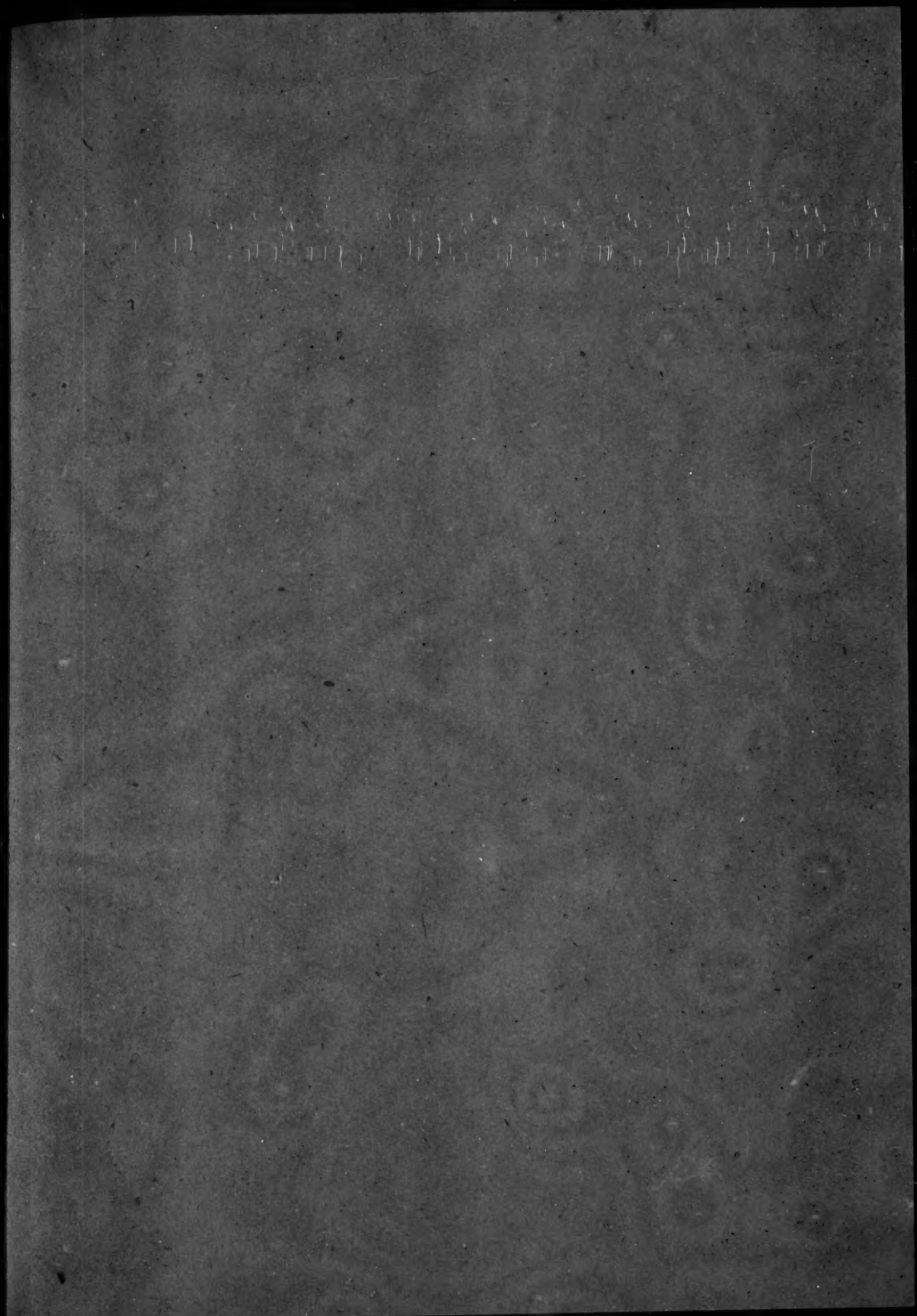
L'Organisation des Os. By P. LACROIX. (Pp. 230; 87 text-figures; 900 fr.) Paris: Masson et Cie; Liége: Éditions Desoer. 1949.

Prof. Lacroix of Louvain has rendered a service to all who are interested in problems of bone-growth by bringing together the results of his researches in accessible form. This monograph is based on communications published between 1941 and 1949, of which twenty-

seven are listed under the author's name in an excellent general bibliography. It discusses the general mode of growth of bones and the role of the periosteum; but the principal chapters are concerned with the nature and function of epiphysial cartilage ('cartilage de conjugaison') with the experimental evidence for the presence of an organizing substance which he proposes to call 'ostéogénine'. The author has come to the conclusion that bone-growth is essentially an induction phenomenon and himself states that conception as the basis of this work.

The book, paper-covered, is well printed on good paper, illustrated mainly by the author's own original figures, and can be recommended as an excellent introduction to the general subject with which it deals.

J. C. BRASH



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